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A COMPARISON OF THE DISTRIBUTION
OF THE INTESTINAL PROTOZOA OF THE
NORWAY RAT, WOOD RAT, AND GUINEA PIG
WITH REFERENCE TO
THE HYDROGEN ION CONCENTRATIONS
AS DETERMINED BY THE GLASS ELECTRODE

BY

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1. The *Norway rat* is omnivorous, with the cardiac and pyloric parts of the stomach very distinct. The length of the small intestine is 101–116 cm.; the caecum, 2–3 cm.; and the large intestine, 18–32 cm.; total, 123–139 cm. There is a well-marked duodenal loop 10 cm. in length. About 20 Peyer's patches are present throughout the small intestine. The relative lengths of the parts of

TABLE 1
RELATIVE LENGTHS OF PARTS OF THE INTESTINE

Animal.....	Norway rat	Guinea pig	Wood rat
Length of small intestine.....	101-116 cm. (108)	152-182 cm. (165)	48-52 cm. (50)
Length of caecum....	2-3 cm. (2.5)	(10-16) cm. 13	8.5-11.1 cm. (10)
Length of large intestine.....	18-30 cm. (20)	84-91 cm. (85)	70-94 cm. (82)
Total.....	123-139 cm. (131)	(245-261) cm. 258	132-152 cm. (142)
Relative length, per cent.....	Small intestine 82.6 Caecum..... 1.9 Large intestine 15.3	63.9 5.0 32.9	35.2 7.7 57.7
Ratio of length of intestine to body length.....	1 : 6	1 : 10	1 : 8
Weight of animal....	200-400 gm.	800-1200 gm.	200-250 gm.
Musculature and connective tissue.....	Well developed	Poorly developed No <i>muscularis mucosa</i>	Well developed
Locality and number of Peyer's patches.....	20 scattered throughout small intestine	Few at beginning of colon	3-4 in small intestine
Ileocaecal valve.....	Poorly developed	Very muscular	Well developed
Brunner's glands.....	No continuity between them and pyloric glands	Continuation of pyloric glands	Not known

the intestinal tract, as shown in table 1, closely resemble those of the cat and dog, which are more strictly carnivorous, and differ greatly from those of the guinea pig and rabbit, which are strictly herbivorous. The caecum of the rat is relatively small and simple, in contrast to the large, sacculate caecum of the guinea pig and wood rat; the large intestine, however, is sacculate. Bayliss (1927) states that there is an alkaline secretion from the walls of the caecum of the *Norway rat* which neutralizes the acid formed by fermentation. Elliot and Barclay-Smith (1904) state that reverse peristalsis is a normal colonic

movement in the rat. The food is thus forced back into the caecum, where contractions push the material again into the colon. These movements facilitate mixing and absorption and transference of Protozoa. They also state that the pH of the material in the caecum and proximal colon was very similar, since these two regions are continually exchanging contents. This is in agreement with our results (Kofoid, McNeil, and Bonestell, 1933) in which the pH of the caecal contents was 6.75 and of the colonic contents 6.77.

2. The *guinea pig* is herbivorous and the cardiac and pyloric parts of the stomach are less distinct than in the rat. The length of the small intestine is 152–182 cm.; the caecum, 12–14 cm.; and the large intestine, 84–91 cm.; total, 258 cm. There is a well-marked duodenal loop, and a very muscular ileocaecal sphincter. The intestine, according to Oppel (1897), lacks a *muscularis mucosa*, and the musculature and connective tissue are poorly developed; two-thirds of the whole villous substance is epithelium. Apparently the glandular efficiency is quite low, but is compensated for by a great increase of surface. According to Schwarz and Rasp (1926), the saliva of guinea pigs contains an active amylase.

Besides the enzymes formed by the animal, food enzymes are of some importance in the herbivores. In the caecum and large intestine cellulose is split by the fermentative action of bacteria to fatty acids. The caecum itself is therefore large and sacculate and shows definite spiral twisting which assists in peristalsis. Large absorbent flaps of mucous membrane extend into the interior of the caecum. Elliot and Barclay-Smith (1904) point out that the chief enlargement, however, is not of the caecum, but of the proximal colon.

3. The *wood rat* is herbivorous and the cardiac and pyloric parts of the stomach are very distinct. The length of the small intestine is 48–52 cm.; the caecum, 8.5–11.1 cm.; and the large intestine, 82 cm.; total, 142 cm. The ileocaecal sphincter is more muscular than that of the Norway rat, and less so than that of the guinea pig. Peyer's patches are found in the small intestine, but the number (3–5) is small. The parotid glands of this genus are particularly rich in ptyalin, according to Howell (1926).

The small intestine is only 35.2 per cent of the total length of the intestine. This percentage is much the lowest found by us in any laboratory or domestic animal. This could be accounted for in two ways: (1) the parotid glands and those of the small intestine are particularly efficient, and (2) the normal diet of the animal contains an exceptionally large amount of cellulose. Since cellulose is digested by bacterial action in the caecum and colon we find a relatively great development of these two parts. The caecum is 8 per cent of the total length, and the large intestine nearly 60 per cent. The caecum is very sacculate, as is also the large intestine. There is a valve in the colon 40 mm. posterior to the caecum.

DISTRIBUTION OF PROTOZOA

Trichomonas—

The genus *Trichomonas* was found in every individual of all three species of host—most plentifully in the Norway rat and least plentifully in the guinea pig. There was no indication in either the wood rat or the Norway rat that *Trichomonas* was pathogenic, although it is frequently present in enormous numbers. However, in the guinea pig red blood corpuscles have often been found ingested by these flagellates. This is a further indication that *Trichomonas caviae*, at least, is not a harmless commensal. It has been argued that any species of *Trichomonas* would probably ingest red blood corpuscles if available. Possibly this is true of culture forms where great numbers of corpuscles are placed directly in contact with the flagellates in a more or less abnormal environment. But if the flagellates are found engorged with corpuscles *in vivo*, it would seem to be an index of a certain degree of pathogenicity. Possibly it is because the musculature and connective tissue of the guinea pig are so poorly developed that the Protozoa are able to penetrate the tissue and produce ulceration, as noted by Wenyon (1925).

In all three species of mammals there is great variation in size of *Trichomonas*. Indeed, it is a subject of controversy, whether, in the Norway rat, there are one, two, or three species of *Trichomonas* present. Wenrich (1930) believes that there are three species, namely, *T. muris*, *T. parva*, and *T. minuta*, and that *T. minuta* has only half the chromosome number of *T. muris*. Ratcliffe (1929) states that the species can be separated by culture methods, but in our experience there is equally great variation in size of the culture forms of each.

The *Trichomonas* from the wood rat resembles *T. caviae* more than *T. muris*. It has a less rigid ectoplasmic layer and a longer axostyle than *T. muris*. The *Trichomonas* from the guinea pig appears to be one species (*T. caviae*) in spite of a very great size range (3.5–20 μ). *Trichomonads* from all three species of mammals have been successfully cultured on Lockes-egg-blood, Lockes-egg-albumin, and Lockes-egg-serum. The last with sheep serum 1:8 gave most satisfactory results.

The distribution of *Trichomonas* in the intestinal tract of the three mammals is quite different. Here again, as in morphology, the species from the wood rat resembles the one from the guinea pig more closely than the one from the Norway rat. Likewise, the pH of the intestinal tract of the wood rat and guinea pig is somewhat similar.

In the Norway rat there is definite correlation between the pH and the presence of *Trichomonas*. In the small intestine it occurs only in the most alkaline part of the ileum at a pH of 6.75–6.82. Above this point the pH is lower (6.75–6.38) and *Trichomonas* is absent. In the wood rat the pH of the entire small intestine has a range of 6.6–7.5 and *Trichomonas* occurs in the upper duodenum in 70 per cent of the animals examined. In 50 per cent it occurs up to

TABLE 2
DISTRIBUTION OF TRICHOMONAS

Wood rat				Guinea pig						Norway rat							
Animal	Inches below stomach				Animal	Inches below stomach						Inches below stomach					
	1	5	10	18		1	5	18	30	42	60	1	6	12	18	24	30
1	-	-	+	+	1	-	-	+	+	+	+	1
2	-	+	+	+	2	-	-	+	+	+	+	2
3	+	+	+	+	3	-	-	+	+	+	-	3
4	+	+	+	+	4	+	+	+	+	+	+	4
5	+	+	+	+	5	+	+	+	+	+	+	5
6	+	+	+	+	6	+	+	+	+	+	+	6
7	+	+	+	+	7	-	-	-	-	-	+	7
8	+	+	+	+	8	-	-	-	-	-	+	8
9	-	+	+	+	9
10	+	+	+	+	10
11	-	-	+	+	11
12	+	+	+	+	12
13	-	-	+	+	13
14	-	-	-	+	14
15	-	-	+	+	15
16	+	+	+	+	16
...	17
...	18
...	19
...	20
Per cent	56	63	69	100	Per cent	38	38	63	63	63	87½	Per cent	5	50

within one-half inch of the pyloric valve. In the Norway rat only large forms are found above the ileocaecal sphincter, but in the wood rat both small and large forms are present.

In the guinea pig the pH of the entire intestinal tract is high (7.0–7.7) and *Trichomonas* occasionally occurs in the upper duodenum (25 per cent of the animals examined).

Trichomonas is thus found in the small intestine in the following regions:

(1) In the *Norway rat*, in the ileum in 40–50 per cent of the animals examined, occasionally in the upper ileum;

(2) in the *wood rat*, in the upper duodenum in 56 per cent of the animals, in the jejunum in 70 per cent, and the lower ileum in 100 per cent;

(3) in the *guinea pig*, in the upper duodenum in 38 per cent of the animals, in the jejunum in 63 per cent, and in the lower ileum in 88 per cent.

These results indicate that some of the Protozoa which are swallowed establish themselves in the small intestine, even as far anteriorly as the duodenum when conditions are favorable, as in the wood rat and guinea pig.

Other flagellates—

Chilomastix was also found in all three species of mammals, but only in the caecum and colon, and never above the ileocaecal valve. It was found in the Norway rat in 60 per cent of the animals examined, in the wood rat in 25 per cent, and in the guinea pig in 90 per cent. It was never found in the small intestine. Possibly it is more dependent on the products of digestion in the caecum and colon.

Hexamitus muris was found only in the Norway rat throughout the small intestine and caecum at a pH range of 5.98–7.27.

Guinea pigs from three colonies were examined. In those from one colony *Chilomitus caviae* was found in the caecum and colon. *Embadomonas* was found in the caecum and colon of those from two colonies. A very curious flagellate, *Selenomonas palpitans*, was found in the caecum of guinea pigs from one colony.

We have found *Giardia* in the Norway rat only, although we have examined wood rats from two localities in California and guinea pigs from three colonies. It has been reported from guinea pigs from the eastern United States. In the Norway rat it occurs most plentifully in the jejunum at a pH range of 6.45–6.52. In no adult Norway rat have we ever found motile *Giardia* below the ileocaecal valve.

Amoebae—

These were found only in the Norway rat, in 70 per cent of the animals examined. Motile forms were fairly plentiful in the caecal material at an average pH of 6.8; only once did we find trophozoites in the ileum. So far as they were identifiable, the amoebae were *Councilmania decumani*. Other workers have found a large species of *Endamoeba* as well as *Endolimax* in the caecum of the guinea pig.

Ciliates—

These were found only in the guinea pig, in the caecum and colon. *Balantidium coli* was present, but showed no signs of being pathogenic. Another ciliate resembling *Paraisotricha* was also present.

In the guinea pigs in which ciliates were present the flagellates were appreciably fewer, but the pH was approximately the same. More species of Protozoa are found in the guinea pig than in either of the other rodents, but less plentifully.

Coccidia—

These were found in 30 per cent of the wood rats from San Diego. Two species have been described by Henry (1932), namely, *Eimeria neotomae* and *E. residua*. Oöcysts were found in the jejunum, ileum, and caecum. Apparently the pathogenicity, if any, is very slight. Coccidia are definitely pathogenic for the guinea pig and Norway rat.

SUMMARY

1. *Trichomonas muris* was found in the lower ileum, caecum, and colon of the Norway rat at a pH range of 6.56–7.0. *Trichomonas* sp. from the wood rat was found throughout the small intestine, caecum, and colon at a pH range of 6.72–7.5. *T. caviae* occurred most plentifully in the jejunum, ileum, caecum, and colon at a pH range of 7.2–7.88. It also was found in the duodenum in 38 per cent of the animals studied, at a pH range of 7.2–7.5.

2. *Chilomastix bettencourti* was found in the caecum and colon of the Norway rat at a pH range of 6.4–7.0. *Chilomastix* sp. from the wood rat was found in the caecum and colon at a pH range of 7.0–7.5. *Chilomastix intestinalis* was found in the caecum and colon of the guinea pig at a pH range of 7.6–7.88.

3. *Giardia muris* was found to be most plentiful in the jejunum of the Norway rat at a pH range of 6.45–6.52. It was also found in the duodenum and ileum at a pH range of 6.36–6.7.

4. *Hexamitus muris* was found throughout the small intestine and caecum of the Norway rat at a pH range of 5.98–7.27.

5. *Selenomonas palpitans*, *Chilomitus caviae*, and *Embadomonas caviae* were found in the caecum and colon of the guinea pig at a pH range of 7.1–7.87.

6. *Balantidium* and *Paraisotricha* were found only in the caecum and colon of the guinea pig at a pH range of 7.7–7.87.

7. Amoebae were found only in the caecum and colon of the Norway rat at a pH range of 6.61–7.0.

8. Coccidia were found in the jejunum, ileum, and caecum of the wood rat at a pH range of 6.6–7.3.

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VARIATIONS IN THE CYTOLOGY OF THE BLOOD
OF GECKOS (*TARENTOLA MAURITANICA*)
INFECTED WITH
HAEMOGREGARINA PLATYDACTYLI,
TRYPANOSOMA PLATYDACTYLI, AND
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INTRODUCTION

THE DATA PRESENTED were obtained from lizards, *Tarentola mauritanica mauritanica* Linnaeus, obtained from Spain. One group of lizards arrived about July 1, 1932, and another group about December 1, 1933. Adult individuals only were used.

Thin smears of heart blood, obtained with a paraffin-lined syringe, were made on glass slides. Air-dried films were stained with Wright's, May-Giemsa, and Jenner-Giemsa. The best preparations were mounted in Grubler's euparal-green.

Satisfactory smears were obtained from one animal (case N) with neither Haematozoa nor intestinal worms, from one animal (case H) infected with *Haemogregarina platyductyli* Billet (1900), from one animal (case T) infected with *Trypanosoma platyductyli* Catouillard (1909), and from one animal (case P) infected with *Pirhemocytion tarentolae* Chatton and Blanc (1914, 1916). All the Haematozoa-infected geckos had nematode worms in the large intestine.

The degree of infection with the different blood parasites varied. Case H showed a light infection as noted by the presence in three smears of only eight haemogregarines (pl. 1, fig. 13), one free and the others intra-lymphocytic.

One evenly spread smear of case T covering about one-half the slide contained 111 trypanosomes (pl. 2, fig. 27). Case T also showed a mild *Pirhemocytion* infection although very few albuminoid bodies were present. Contrary to the findings of Chatton and Blanc (1914, 1916), most of the small amoe-

boid forms of the parasite, which were present in more than 75 per cent of the red cells, were not associated with albuminoid bodies. "Paranuclear bodies" of Bremer (Billet, 1904) were also found in association with this parasite (pl. 2, fig. 17). Only one haemogregarine was found in smears from case T.

Smears from case P showed a greater disturbance than that previously observed by Chatton and Blanc (1914, 1916), who reported that 50 to 75 per cent of the red cells contained albuminoid inclusions with which the parasites were always associated. In one count from case P, almost all the normoblasts (488 or 96 per cent of 500 erythrocytes counted) were parasitized, and the younger cells or erythroblasts (18 or 4 per cent) were without *Pirhemoctyon*. Paranuclear bodies were also present. Case P was also infected with trypanosomes, which numbered about 59 per smear in contrast to 111 in case T.

NORMAL BLOOD PICTURE

THE BLOOD PICTURE of this gecko has previously been described by Pappenheim (1909), Werzberg (1911), and Alder and Huber (1923). Werzberg (1911), a student of Pappenheim, distinguished the following cell types: orthochromatic normocytes, polychromatic normoblasts, megaloblasts, orthochromatic microcytes, polychromatic microblasts, spindle cells, mast cells, eosinophils, mononuclear leucocytes, small and large lymphocytes, leucocytoid lymphocytes, and micromyeloblasts.

Alder and Huber (1923) studied the blood and blood-forming tissues. They distinguished erythrocytes, thrombocytes, neutrophil, eosinophil, and basophil leucocytes, myelocytes, and haemocytoblasts. Their leucocytic formula for the gecko is as follows: 73 per cent haemocytoblasts and thrombocytes, 1 per cent myelocytes, 1 per cent neutrophils, 7 per cent eosinophils, and 18 per cent basophils.

The author wishes to point out that no statement of the parasitic infections of the animals was given by the previous workers. The presence of worms and Haematozoa markedly alters the leucocytic formula of the host.

The terms *myeloblast* and *lymphoblast* include types which correspond structurally to these classes of mammalian blood cells. The myeloblast designates a cell with round or oval nucleus which has narrow, fine, interlacing chromatin strands, narrow parachromatin spaces, and several nucleoli. The cytoplasm is very darkly stained and sometimes granulated. These cells are forerunners of the granular leucocytes. The lymphoblast (pl. 1, fig. 10) has a nucleus with structure similar to that of the myeloblast. The cytoplasm is deeply basophilic and usually nongranulated. The micromyeloblasts of Werzberg are classed here as myeloblasts.

The *lymphocytes* are typical in form and are easily recognized (pl. 1, figs. 6, 11). They possess a darkly stained nucleus of broad homogeneous chromatin strands and narrow parachromatin spaces. Sometimes the chromatin is distinctly clumped. The relatively scanty cytoplasm is light or dark blue. Alder and Huber (1923) classify small lymphocytes as haemocytoblasts.

Mononuclear leucocytes or *monocytes* are very large cells with broad, light basophilic cytoplasm and oval or indented nucleus with narrow chromatin strands and narrow parachromatin spaces (pl. 1, fig. 8). The cell sometimes shows fine, azurophilic granules scattered throughout its cytoplasm. Frequently, pigment granules are present in the cytoplasm. This cell corresponds to the lympho-leucocytes of Pappenheim (1909) and the large mononuclear leucocytes of Werzberg (1911). Alder and Huber (1923) fail to mention this type.

Pigment cells have been reported from the blood of reptiles. The author believes that the presence of these cells is to be accounted for either by mechanical transmission of pigment cells from the connective tissue by needle puncture through the pericardial sac or by the carrying of some of the pigment from surrounding membranes into the blood stream where it is phagocytized by monocytes.

The *eosinophil leucocytes* are characterized by a simple, round, oval, indented, or polymorphic nucleus and oxyphilic cytoplasm in which two types of granules are embedded (pl. 1, figs. 2, 4, pl. 2, figs. 19, 20). These are the definitive acidophil crystalloids or granules and the fine amphophil granules. The former usually occur in a tuft or star-like arrangement about a clear area in the center of the cell, and the latter are scattered throughout the cytoplasm. Most of the cells are of the polymorphonuclear type, mononuclear forms being rarely seen. Alder and Huber place the mononuclear eosinophils in the myelocytic category and use the term eosinophil leucocyte for the polymorphonuclear forms. All types with definitive acidophil granules are included in this paper under the term eosinophil leucocyte as Pappenheim and Werzberg have done.

The *basophil leucocyte* has a simple round or oval nucleus of lymphocyte-like structure. The cytoplasm, in which from one to several hundred irregularly shaped, deeply basophilic granules of variable size are found, is faintly oxyphilic (pl. 1, fig. 3). All cells with any definitive granules are classed here as basophil leucocytes. This agrees with the groupings of Pappenheim and Werzberg; it is opposed to the classification of Alder and Huber, who use the term myelocyte for cells with few granules and the term leucocyte for cells with many granules.

Neutrophil leucocytes possess a round, oval, or indented nucleus with narrow, coarse, irregular chromatin strands or clumps and narrow parachromatin spaces. The cytoplasm is basophilic or slightly oxyphilic with small, irregularly outlined, nonrefractile neutrophil granules similar to those found in the cytoplasm of the eosinophils. Sometimes, in the cytoplasm at about the center of the cell or opposite the indentation of the nucleus, there is a small clear area about which are clustered distinct, dark, purplish granules and rodlets in an irregular tuft or star-like arrangement (pl. 1, figs. 7, 9). This cell corresponds to the leucocytoid lymphocytes of Werzberg and the neutrophils of Alder and Huber.

The term *special leucocyte* is here restricted to cell types described from other species of reptiles by Pappenheim (1909) and Werzberg (1911). They

are very large cells with a polymorphic nucleus and oxyphilic or slightly basophilic cytoplasm. In the cytoplasm, there are numerous fine amphophil granules identical with those found in the eosinophils. In addition, they may show one or more dark orange or brownish granules or crystalloids. These are remnants of the tuft of acidophil crystalloids and they were present in most cells of this type (pl. 1, fig. 5). Only rarely were these granule remnants lacking. Alder and Huber did not find this cell type and it was most numerous here in case H.

The *erythrocytes* are elongate, ovoid, centrally bulging disks with a distinctly ovoid nucleus of coarse chromatin strands and small angular blocks, interspersed with narrow parachromatin spaces. In the cytoplasm, there are frequently found one or two paranuclear bodies (pl. 1, fig. 1, pl. 2, fig. 17). The term erythrocyte, as used here, designates orthochromatic normoblasts. This corresponds to the haemoglobinated, nucleated red blood cell or normoblast of mammals. This cell type is identical with Alder and Huber's (1923) erythrocyte and includes Werzberg's (1911) orthochromatic normocytes and microcytes.

The term *erythroblast* here includes all earlier developmental forms of the erythrocytes up to the orthochromatic normoblast (pl. 2, figs. 18, 21, 25, 28). All forms of round or ovoid shape, showing deeply basophilic or polychromatic cytoplasm, are included. The nucleus of these younger cells is usually round or oval and shows many coarser deeply stained chromatin blocks. These are separated by narrow parachromatin spaces. This term includes Werzberg's megaloblasts and polychromatic normoblasts and microblasts.

Thrombocytes are cells of similar shape, but are smaller in size than erythrocytes (pl. 1, fig. 12). In well-fixed preparations, the nucleus is ovoid in shape and characterized by deeply stained, coarse, irregular chromatin strands and clumps, with sparse parachromatin spaces. The cytoplasm is usually visible only at opposite ends of the ovoid nucleus, the cell membrane being pressed to the sides of the nucleus. The cytoplasm is faintly basophilic and often shows one to several bright reddish granules in a finely reticulated meshwork at either or both poles of the cell. In some cells the plasma membrane is very irregular and in others it is evenly contoured. This description agrees with that of Werzberg. Alder and Huber state that the nucleus of the thrombocyte is pycnotic. This is also true of the author's preparations in overstained portions of the smear. Only in preparations where there is little or no clotting and the cells have been rapidly dried, can their typical structure be seen.

Where the thrombocytes are poorly fixed, they may be distinguished from small lymphocytes in the following way. The nucleus of the small lymphocyte is homogeneous and shows more parachromatin, sometimes separating distinct chromatin blocks which stain less deeply than the nucleus of the thrombocytes, the nucleus of the latter being very dense and deeply stained and showing ridges, crenations, or folds. The cytoplasm of the thrombocyte is very lightly stained in contrast to the basophilia of the lymphocytic cytoplasm. It is more fluid and does not resist distortion in smearing nearly as well as the

cytoplasm of the lymphocytes. Besides, the thrombocyte cytoplasm is limited by a distinct, heavily stained, cell membrane which is not present in the lymphocytes. When this membrane is destroyed, the cytoplasm is distributed in string-like projections about the cell, giving the appearance of an irregular fringe.

CHANGES IN THE BLOOD PICTURE WITH HAEMATOTOZOAN INFECTIONS

Changes in the erythrocytes.—The appearance of the red cells in cases N and H is somewhat in contrast to that of cases T and P. In the former the erythrocytes are of about the same size and shape, presenting a very homogeneous aspect as one surveys the smear. Scattered throughout the orthochromatic normocytes, however, are a few erythroblasts (mostly polychromatic normoblasts).

The erythrocytes of case T show almost the same structure as those of case N except that most of the cells have one or two paranuclear bodies and many have the small amoeboid form of *Pirhemocytton* (pl. 2, fig. 17). In a few cells only, albuminoid globules are present and in these cells there is a beginning of the tendency to broaden, the cells becoming more ovoid and the nucleus oval.

The broadening tendency of the erythrocytes is best shown in case P (pl. 2, figs. 14, 15). All the red cells show definitely a more rounded shape with round nuclei as well as the generally spherical albuminoid inclusion and the variable forms of the parasites in the cells. The action of the parasite probably makes the cytoplasmic contents of the red cell more fluid, thus tending toward a more rounded form of the cell. Erythroblasts as well as erythrocytes are infected. Nonnucleated portions of erythrocytic cytoplasm are not rare and amitotic division is an active accompaniment of the parasitic infection as shown by the presence of dumbbell-shaped cells and many cone-shaped nucleated and nonnucleated portions of erythrocytic cytoplasm.

The erythroblasts in the circulating blood of cases N and H are of the polychromatic normoblast type and probably represent a normal condition. In case T, no erythroblasts of the type noted in either of the other lizards were found. Case T showed a greater proportion of pycnotic normocytes (pl. 2, fig. 16) than was shown in either of the other animals, clearly indicating a degenerative tendency as far as the erythrocytic series is concerned.

The opposite relation is shown in case P, since the blood is decidedly in an active state of regeneration. Erythroblasts (pl. 2, figs. 18, 21, 25, 28) are present in considerable numbers and all transitions from the basophil erythroblast (pl. 2, fig. 28) to the mature normocyte (pl. 2, fig. 14) occur. Whether or not this regenerative activity is wholly caused by the parasites is uncertain, since Jolly (1923) points out that such changes occur in reptiles after periods of dormancy. It is possible that limitations of the activity of the lizards imposed by the limited space of the cages may have some effect upon this condition.

Changes in the leucocytes.—The following table of differential leucocyte counts shows the differences in the proportions of cells present. Counts of 1500 white cells for each animal were made from three different smears (500 cells per smear) and the average percentages calculated. The following abbreviations are used: eosino., eosinophils; baso., basophils; neutro., neutrophils; lympho., lymphocytes; blasts., myeloblasts and lymphoblasts; mono., monocytes; pig. c., pigment cells, and sp. l., special leucocytes.

TABLE 1
DIFFERENTIAL LEUCOCYTE COUNTS

Animal	Eosino.	Baso.	Neutro.	Lympho.	Blasts.	Mono.	Pig. C.	Sp. L.
Case N	57.00	19.86	7.00	10.20	4.73	0.60	0.26	0.33
Case H	40.80	27.66	16.53	9.86	1.26	0.66	0.46	2.73
Case T	27.40	37.06	24.30	5.60	3.60	1.20	0.33	0.46
Case P	18.93	35.80	28.40	11.46	3.20	1.46	0.73	0.00

The eosinophil leucocytes show a steady decrease in number from case N to case P and the basophil leucocytes show a steady increase in number from case N to case T. There is an increase in the number of neutrophils from case N to Case P, but the number of lymphocytes remains about the same.

The eosinophils of case N are typical (pl. 1, fig. 2). They have a tuft of acidophil crystalloids in the central part of the cell, flanked on one side by the highly polymorphous nucleus and on the other by the amorphous mass of amphophilic granular material in the oxyphilic cytoplasm. In case P, the eosinophil granulation is less compact, being more spread out in the cell (pl. 2, fig. 20), and several degenerating cells were found (pl. 2, figs. 23, 24).

The basophils of case N are for the most part densely packed with many dark, purplish granules. In case P many cells have relatively few granules (pl. 2, figs. 22, 26).

Thus, in all three haematozoan infections, there is a definite and differential response of different types of white cells to the different stimuli as represented by the blood-inhabiting Protozoa. Another factor, not, however, differential among the hosts of the blood-inhabiting Protozoa, was the presence of nematode worms in the digestive tract.

DISCUSSION

THE SPECIAL LEUCOCYTE has been reported by Pappenheim (1909) and Werzberg (1911) from other reptiles, but not from this gecko. The cell here seems to be nothing more than an eosinophil which has lost its tuft of acidophil granules. The existence of many transition stages from cells with few granules to nongranulated forms suggests this interpretation. Furthermore, the cytoplasm of the special leucocytes and eosinophil leucocytes is identical, both showing a light oxyphilic paraplasm with a meshwork of amphophil granular material. The nuclei of both cells are the same in size, shape, and structure and the cells themselves are approximately alike in size.

Of the previous investigators who have counted the white cells of reptiles, Sabrazès and Muratet (1924) found in a haemogregarine infection of a lizard (*Lacerta muralis*) a decreased number of eosinophils (about 18 per cent), the mast cells remaining about the same. Babuder (1930) found, in a haemogregarine-infected lizard (*Lacerta muralis*), a decrease in the number of eosinophils in comparison with counts from most of the noninfected animals (0 to 11 per cent), but the number of basophils remained about the same. However, in *Lacerta viridis*, he reported one animal infected with haemogregarines which showed an increase of neutrophils over the noninfected lizards of from 30 to 40 per cent, a decrease of eosinophils of from 5 to 47 per cent, and about the same number of basophils. In the present investigation, case H shows a 9 per cent increase of neutrophils, a 17 per cent decrease of eosinophils, and an 8 per cent increase of basophils.

Loewenthal (1928, 1930), who has investigated the blood of several different reptiles, gives some general figures for the different cell types as follows: 34–77 per cent lymphocytes, 8–15 per cent mononuclears (small and large), 0–22.5 per cent neutrophils, 5–8 per cent and 22–24 per cent eosinophils, and 0.9–2 per cent and 8–25 per cent basophils.

A more extensive study of the leucocytic formula of reptile blood is that published in 1930 by Babuder, whose figures show the following ranges for the different cell types: 5–92 per cent lymphocytes, 0–16 per cent monocytes, 0–53 per cent neutrophils, 0–59 per cent eosinophils, and 0–22 per cent (one count, 72 per cent) basophils. The quantitative diversity of these figures shows the extreme numerical variability among different species for the cellular constituents of reptile blood. Such changes seem to indicate that active agents bring about altered conditions in the body metabolism of the animal. Three important factors tending to extend these ranges of variation are Haematozoa, helminth infections, and temperature.

SUMMARY

1. The normal blood picture of the gecko comprises erythrocytes (orthochromatic and polychromatic normoblasts), thrombocytes, eosinophil, basophil, and neutrophil leucocytes, lymphocytes, monocytes, myeloblasts, and lymphoblasts.

2. The destructive action of an erythrocyte-invading parasite, *Pirhemocytton*, stimulated active erythroblast formation in one gecko. All stages of development from the basophil erythroblast to the orthochromatic normoblast were found in the circulating blood.

3. Decreased number of granules per cell, as well as lessened regularity of distribution and arrangement of granules in the eosinophil and basophil leucocytes, was noted in a gecko infected with *Pirhemocytton*.

4. A gecko free from intestinal worms and haematozoa showed the following leucocytic formula: 57 per cent eosinophils, 19 per cent basophils, 7 per cent neutrophils, and 10 per cent lymphocytes.

5. A gecko infected with haemogregarines showed changes in percentages

as follows: a 16 per cent decrease of eosinophils, a 7 per cent increase of basophils, a 9 per cent increase of neutrophils, and no change in the number of lymphocytes.

6. A gecko infected with trypanosomes showed changes in percentages as follows: a 29 per cent decrease of eosinophils, a 17 per cent increase of basophils, a 17 per cent increase of neutrophils, and a 4 per cent decrease of lymphocytes.

7. A gecko infected with *Pirhemocytion* showed changes in percentages as follows: a 38 per cent decrease of eosinophils, a 15 per cent increase of basophils, a 21 per cent increase of neutrophils, and about the same number of lymphocytes.

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EXPLANATION OF PLATES

All figures were drawn with the aid of a camera lucida, $\times 1900$. The following abbreviations are used: H, infected with haemogregarines; T, infected with trypanosomes; and P, infected with *Pirhemocytos*. The illustrations for case H are typical of the blood of case N (noninfected gecko) with the exception of figure 13. Figures 1, 5, 8, 10, 11, 12, 16, 18, 26, and 28 were stained with May-Giemsa. All others were stained with Jenner-Giemsa.

Plate 1

- Fig. 1. H, normal erythrocyte (orthoehromatic normoblast) with paranuclear bodies.
- Fig. 2. H, normal eosinophil leucocyte showing typical arrangement of granules.
- Fig. 3. H, normal basophil leucocyte showing densely packed granulation.
- Fig. 4. H, normal eosinophilic myelocyte.
- Fig. 5. H, normal special leucocyte showing the remains of one eosinophil crystalloid.
- Fig. 6. H, normal large lymphocyte.
- Fig. 7. H, normal neutrophil leucocyte.
- Fig. 8. H, normal monocyte.
- Fig. 9. H, normal neutrophil leucocyte.
- Fig. 10. H, normal primitive lymphocyte, perhaps a lymphoblast.
- Fig. 11. H, normal small lymphocyte.
- Fig. 12. H, normal thrombocyte.
- Fig. 13. H, intralymphocytic *Haemogregarina platydactyli*.

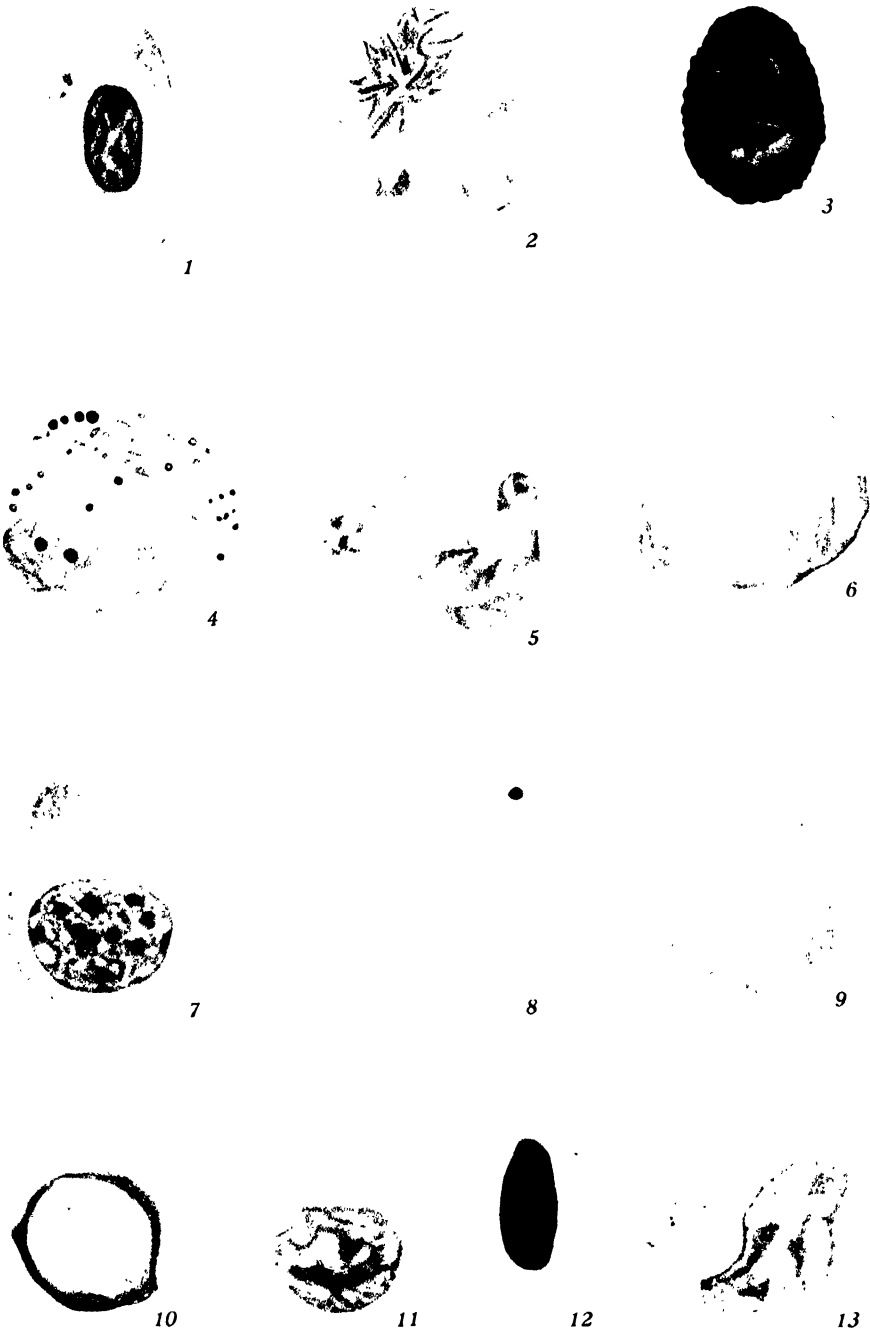


PLATE 2

PLATE 2

- Fig. 14. P, erythrocyte showing large albuminoid body and parasite *Pirhemocyon tarentolae*. Note the more rounded appearance and the evidences of nuclear degeneration.
- Fig. 15. P, round form of infected erythrocyte.
- Fig. 16. T, degenerating erythrocyte.
- Fig. 17. T, erythrocyte showing small amoeboid form of *Pirhemocyon tarentolae* associated with a small vacuole. Note the paranuclear body in the upper right part of the cell.
- Fig. 18. P, polychromatic erythroblast.
- Fig. 19. P, eosinophilic myelocyte.
- Fig. 20. P, eosinophil leucocyte showing indefinite scattered arrangement of the crystalloids typical of these cells in animals parasitized with *Pirhemocyon*.
- Fig. 21. P, polychromatic erythroblast.
- Fig. 22. P, basophil leucocyte (myelocyte).
- Fig. 23. P, degenerating eosinophil.
- Fig. 24. P, degenerating eosinophil.
- Fig. 25. P, basophil erythroblast.
- Fig. 26. P, basophil leucocyte.
- Fig. 27. T, *Trypanosoma platydaetyli*.
- Fig. 28. P, basophil erythroblast.



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THE CYCLE OF TRYPANOSOMA CRUZI IN TISSUE CULTURE OF EMBRYONIC HEART MUSCLE

BY

C. A. KOFOID, F. D. WOOD, AND E. McNEIL

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THE CYCLE OF *TRYPANOSOMA CRUZI* IN TISSUE CULTURE OF EMBRYONIC HEART MUSCLE

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C. A. KOFOID, F. D. WOOD, AND E. McNEIL

THIS REPORTS the cyclic growth of *Trypanosoma cruzi* in tissue cultures of embryonic heart of rat and mouse.

The tissue cultures were made from 14- to 16-day rat and mouse embryos. They were grown in plasma-embryo extract medium on 7.4×4.5 cm. slides having a depression 4 mm. deep. They were incubated 24 hours at 37° C. before being inoculated with culture forms, in semisolid blood agar medium, of the California strain of *T. cruzi* from the wood rat. Seven sets of cultures of 8 slides each were prepared, 3 sets with mouse heart and 4 with rat heart. One slide from each set was kept as a control. The cultures were examined at frequent intervals during the first 24 hours after inoculation, and at least twice daily thereafter for 6 days. Stained preparations, both of smears and of sectioned tissue, were made daily for control of observations on the living material.

Some of the crithidial culture forms used in the inoculum survived at 37° C. in the surrounding medium in the tissue cultures as long as the experiment lasted (maximum period of 7 days) and apparently continued to multiply therein, as shown by the presence of numerous predivision forms with two flagella and of rosettes. In the first 24 hours there was a decrease in the number of trypanosome forms in the culture medium, these seemingly being the forms which entered the tissues. The trypaniform type was always less plentiful in the culture tubes than the crithidial type, and after 48 hours in tissue culture the free-swimming trypanosomes were very rare or lacking. At 24 hours trypanosomes and many crithidias could be seen adhering, either by the posterior end or by the flagellum, to the muscle tissue, fibroblasts, and macrophages. Although several of the adherent forms were watched for 20 minutes under the microscope, actual penetration of the cells was not observed.

The opacity of the muscle made it difficult to determine what was taking place within it. However, the macrophages, fibroblasts, and extracellular parasites could be observed easily. At 24 hours, many macrophages contained from one to several still actively motile flagellates. Because of their motility, it was difficult to determine the number within a cell and whether they were crithidial or trypanosome stages. On the third day, granular debris from degenerating macrophages was observed to contain wriggling flagellates.

No flagellates were ever seen in the fibroblasts, either in living or in stained material, although many attached themselves to the exterior of these cells.

Trypanosomes of the type usually found in the circulating blood were first seen on the fifth day after inoculation. They were at first localized as though emerging from the massed leishmaniform phases. These emerging flagellates are generally shorter and almost metabolic in their movements, as contrasted with the long, slender metacyclic forms which occur in the culture tube and

the insect vector. These blood forms were often found in loosely associated groups in the plasma surrounding the heart muscle from which they had recently emerged. The time of emergence of these forms from the tissues agrees with the generally accepted opinion (Brumpt, 1927; Dias, 1932) that in the infected mammal it requires a minimum of 4 or 5 days for the infective stages to enter the tissues, multiply, and reappear in the blood as trypanosomes.

In the sectioned material parts of the plasma clot containing macrophages frequently adhered to the periphery of the tissue. In a 24-hour preparation stained in iron haematoxylin, macrophages containing one or two parasites were numerous. The outline of the cytoplasm of the parasites was not distinctly differentiated from that of the macrophages, but the rodlike parabasal bodies indicated clearly that they were in the crithidia or leishmania stage, probably the former, since in living 24-hour cultures comparable flagellates were motile.

One section of cultured muscle showed a crithidial stage in the process of entering a macrophage. The posterior end of the parasite was within the cell while the flagellate end was still free. Although these crithidial stages are supposed not to be infective, it is obvious here that they enter, or are ingested by, macrophages, and that they may survive the death of the cell. Since these ingested crithidial stages survive at body temperature, perhaps they eventually develop into infective forms, either within or outside of body cells.

Groups of typical leishmaniform bodies were first seen in sectioned heart muscle from culture on the fourth day after inoculation. The groups varied in number from two to fifty. The type in any one group was approximately the same in all individuals. Some groups were typical, with no flagella, while others were of the stubby crithidial type, with short flagella. A few groups of very slender trypanosomes with round or oval parabasals were noted.

From the foregoing it is clear that the development of *T. cruzi* in tissue cultures of heart muscle is the same, with respect to both time and stages of its cycle, as its development in the body of the living vertebrate host.

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DINOFLAGELLATA OF THE SAN FRANCISCO REGION

I. ON THE SKELETAL MORPHOLOGY OF TWO NEW SPECIES, GONYAULAX CATENELLA AND G. ACATENELLA

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W. FOREST WHEDON AND CHARLES ATWOOD KOFOID

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BY

W. FOREST WHEDON AND CHARLES ATWOOD KOFOID

(Contribution from the George Williams Hooper Foundation for Medical Research, San Francisco, and the Department of Zoology, University of California, Berkeley)

INTRODUCTION

STUDIES OF THE NATURE and possible cause of mussel poisoning which have been undertaken in the past several years have shown that the food materials of the animals affected, consisting of dinoflagellates, diatoms, and detritus, hold promise of a possible solution of the source of the poisoning. It has been found also that the mussels *Mytilus californianus* and *M. edulis*, when found on the open ocean beaches, and the crustacean *Emerita analoga*, all possessed the toxic substance in their digestive diverticula and that they were dinoflagellate feeders. While the studies were in progress, the two species described in this paper were seen to appear in large numbers coincidentally with the period of greatest poison. However, the discussion of a possible relationship between certain Dinoflagellata and the poison will be dealt with in a later contribution.

Gonyaulax catenella, sp. nov.

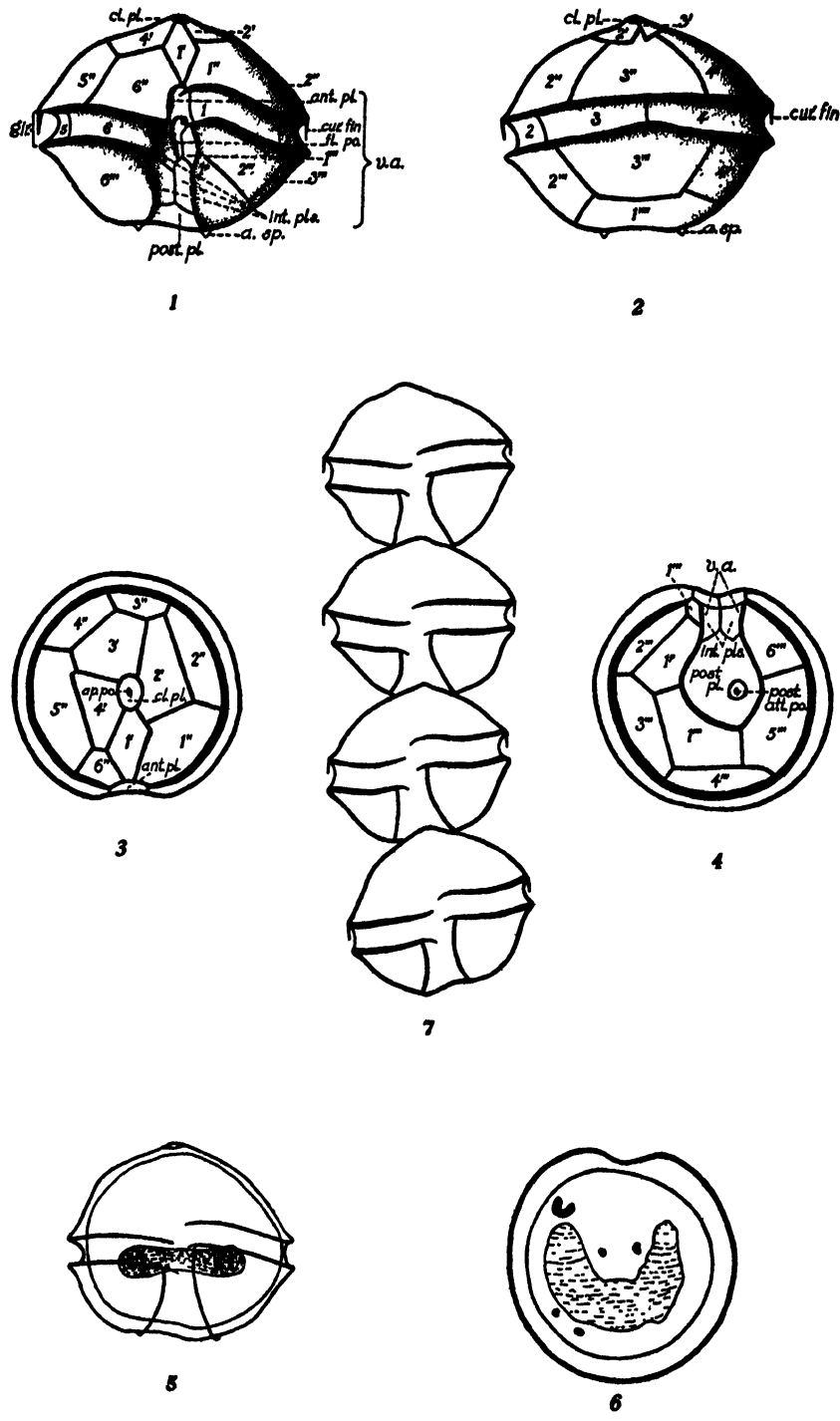
Diagnosis.—A small species with the general form of a *Peridinium* and the plates of the genus *Gonyaulax*; plate formula 4', Oa, 6'', 6''', 1^p, 1''''; usually two short antapical spines; theca free from markings; fission with chain formation; length 30 to 37 (32) μ , transdiameter 37 to 49 (42) μ ; in coastal plankton off San Francisco.

Description.—The *body* is rounded, dorsoventrally flattened, its length 0.71–0.88 of the transdiameter. The dorsoventral diameter is nearly equal (0.70–0.80) to the transdiameter. The *epitheca* and *hypotheca* are nearly equal in altitude. Both shoulders of the *epitheca* are convex, the right one more so than the left. The left side of the *hypotheca* is convex, whereas the right is often slightly concave. There is no apical horn.

The *girdle* (*gvr.*, fig. 1) is equatorial, descending, displaced distally 1 girdle width, without overhang, and in some animals shows a slight constriction between *girdle plates* 3 and 4 (fig. 2). The furrow is deeply impressed, with scarcely salient ridges and no lists. The ends are slightly curved posteriorly.

In dorsal and ventral views many individuals are seen to possess a small *curtain fin* (*cur. fn*, fig. 1), which hangs from the anterior girdle ridge. It measures approximately 3 μ in width.

The *ventral area* (*v. a.*, fig. 1) is exceptionally open and slightly indents the *epitheca*. Its width at the distal posterior ridge varies from 1.0 to 1.5 girdle widths. In this same region



the ventral area, which forms the *longitudinal furrow*, is deeply impressed. The *posterior plate* (*post. pl.*, figs. 1, 4) only slightly impresses the hypotheca and flares posteriorly at the antapex to about 3 girdle widths, the right side flaring more than the left.

Antapical spines (*a. sp.*, figs. 1, 2) were not always present, their presence apparently being restricted, primarily, to the single and older individuals, although in a very few of the animals studied, the posterior individual of a chain did possess very short spines. They arise, one from the margin of the right antapex of the posterior intercalary plate (fig. 1) and the other from the left antapex of the postcingular plate 5''.

The plate formula is 4', Oa, 6'', 6'', 1'', 1''' (figs. 1, 2). *Apical 1'* (figs. 1, 3) is about 0.8 of a girdle width across and elongated. *Apicals 8'* and *4'* (fig. 3) are elongated in the transverse direction, and *8'* (fig. 3) in the longitudinal. *Precingular 6''* (fig. 1) is quadrangular and *postcingular 1'''* (fig. 1) is very small, inconspicuous, and rectangular in shape. The *posterior intercalary 1''* (figs. 1, 4) is much wider than long and is bounded by *postcingular 8''* (fig. 4) and the *posterior plate* of the ventral area. Two *intermediate plates* (fig. 1) of the ventral area of like size are easily distinguished, extending from the distal end of the girdle posteriorly about 1.5 girdle widths.

The *surface* is smooth, nonporulated, and lacks any markings except very faint suture lines. The *anterior attachment pore* (*ap. po.*, fig. 3) is found in the closing plate (*cl. pl.*, fig. 3) of the apex. In some of the individuals studied the *posterior attachment pore* (*post. att. po.*, fig. 4) could be seen in the posterior plate of the ventral area near *postcingular plate 5''*. There are no pores along the girdle and no fin on either margin of the ventral area.

Remarks.—No definite fission line could be found either in chains or in isolated individuals. Asexual reproduction is accomplished by repeated fission with the formation of chains, as far as is known. No cysts were found. Protoplasmic continuity in the chains is accomplished by a slender strand of cytoplasm, in much the same manner as that demonstrated in *Ceratium*. Chain formations with two and four individuals (fig. 7) in a chain are exceedingly common during the period of maximum numbers; and, upon several occasions, as many as eight individuals in a chain have been noted. A marked characteristic of this species, though it has nothing to do with the process of multiplication as far as we have been able to ascertain, is the tendency for the epitheca and hypotheca to separate along the anterior ridge of the girdle in the region of the longitudinal furrow.

EXPLANATION OF FIGURES

Gonyaulax catenella

Fig. 1. Ventral view of theca showing plates. $\times 840$.

Fig. 2. Dorsal view of same. $\times 840$.

Fig. 3. Apical view of epitheca showing plates. $\times 840$.

Fig. 4. Antapical view of hypotheca showing plates. $\times 840$.

Fig. 5. Ventral view of theca showing shape, relative size, and position of the nucleus. $\times 840$.

Fig. 6. Antapical view of same. $\times 840$.

Fig. 7. Four individuals in chain, ventral aspect. $\times 680$.

ABBREVIATIONS

1-6—girdle plates.
1'-6'—apical plates.
1''-6''—precingular plates.
1'''-6'''—postcingular plates.
1''''—antapical plate.
gir.—girdle.
1''—posterior intercalary.

ant. pl.—anterior plate of ventral area.
ap. po.—apical pore.
a. sp.—antapical spine.
cl. pl.—closing platelet.
fl. po.—flagellar pore.
int. pls.—intermediate plates of ventral area.

post. pl.—posterior plate of ventral area.
cur. fin.—curtain fin.
v. a.—ventral area.
post. att. po.—posterior attachment pore.

The *cell contents* are very dense and vary in color from a deep yellow-green to an orange-brown. The color is usually the same in all individuals at the same time and has been observed to change at the same locality from one to the other in a very short period of time, thus demonstrating a susceptibility to external changes. Chromatophores are numerous and densely packed, and oil droplets are seen lying in the center of the cell near the nucleus. Starch granules, demonstrated by the iodine test, are comparatively few in number. The *nucleus* (figs. 5, 6) is large and appears as a brownish, very granular, U-shaped body in unstained material. It lies just inside of the girdle, in the same plane, and with the open end of the U always on the ventral side. The nucleus is easily stained with Heidenhain's haematoxylin following fixation with Flemming's fluid.

Dimensions.—Sixteen individuals were measured. Length (exclusive of spines), 30–37 (32) μ ; transdiameter, 37–49 (42) μ ; dorsoventral diameter, 28–35 (30) μ ; width of the girdle, 5 μ ; length of the spines, 2 μ . Individuals in a given chain have identical measurements.

Distribution.—Taken from Pacific waters off the coast of northern California and Oregon. Water samples containing this form have been collected at Pescadero, Half Moon Bay, Montara, Mussel Rock, all near San Francisco, and Ocean Beach at San Francisco, and Wright's Beach near the mouth of the Russian River in California, and at Port Orford, Bandon, and North Bend (Coos Bay) in Oregon. It is a neritic species and appears in water samples intermittently throughout the year. The time of maximum appearance in 1932 at San Francisco (see graph, fig. 16) was June 27. At the same station in 1933, the maximum number appeared on July 13. The 1934 maximum was recorded on June 10. The duration of these maximum periods was approximately a month except in 1932, no data being available prior to June 17. The temperature range for this form is 9.5° C to 16.0° C, the maximum numbers appearing in samples when the water temperature is between 14.0° and 15.0° C.

Comparisons.—This species closely resembles *Gonyaulax catenata* (Levander), described (1894a) as *Peridinium catenatum*, in appearance and method of chain formation. *Gonyaulax series* Kofoid, a tropical species, also resembles *Gonyaulax catenella* in the fact that it forms chains, but not in the method of formation.

Gonyaulax catenella differs from *Gonyaulax catenata* (Levander) in the size, shape, and position of the apical plates. In the latter species the four apicals are smaller and more irregular in shape. Apicals 2' and 4' in *G. catenata* are elongated in the longitudinal direction and 3' in the transverse direction. This arrangement is exactly the opposite of that seen in *G. catenella*. Apical 1' in both species is of much the same shape, but in Levander's species it is proportionately smaller. *G. catenella* is without anterior intercalary plates, whereas in *G. catenata* there are four. The anterior attachment pore, in the latter species, lies in apical 3' near the apex, but in *G. catenella* the pore is in the apex itself. The posterior pore in both species is in approximately the same position. The posterior plate and antapical plate of *G. catenata* are much wider and shorter than the same plates in the species described in this paper.

Postcingular 1''' of *G. catenata* is much more conspicuous and somewhat larger than the same plate in the latter species. In this species it is more like the same plate in other species of *Gonyaulax*.

Both species are small and shaped like a *Peridinium*, but *Gonyaulax catenella* is more rotund than *Gonyaulax catenata*. The latter is much more flattened dorsoventrally and the proportions of the epitheca and hypotheca are different. The epitheca and hypotheca of *G. catenella* are nearly equal, whereas in *G. catenata* the epitheca exceeds the hypotheca slightly. The latter species has no curtain fin on the girdle, but does have several pores along the ridges of the girdle.

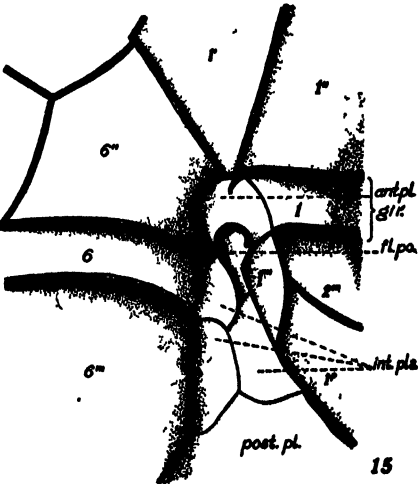
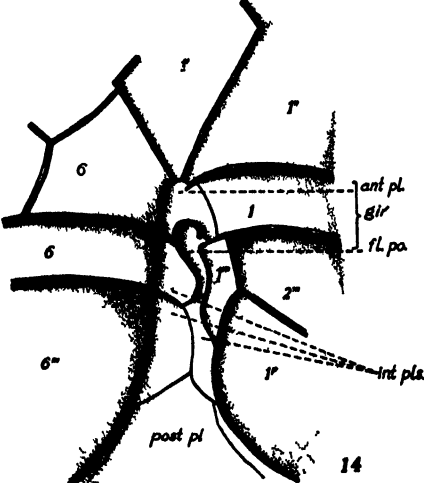
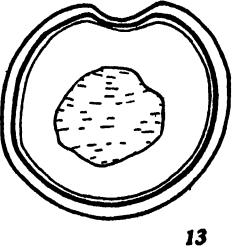
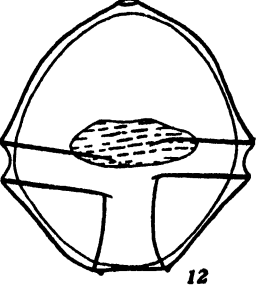
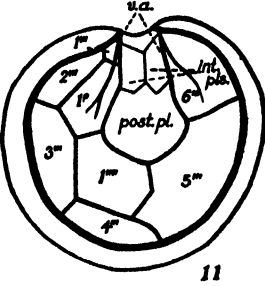
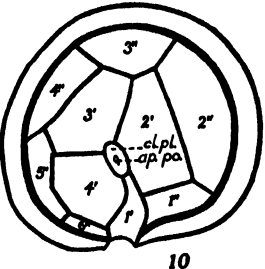
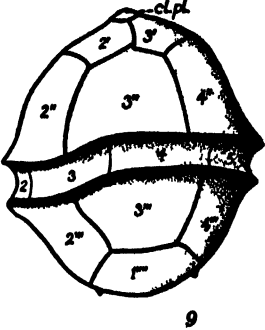
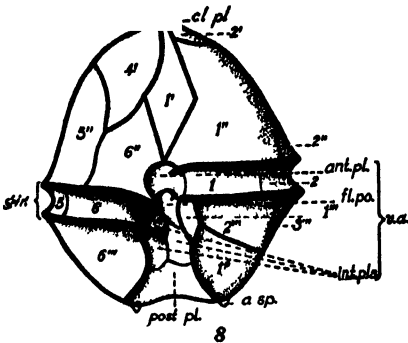
The surface of *Gonyaulax catenata* is reticulate, whereas the surface of *Gonyaulax catenella* is smooth and free from pores. There is a marked difference in the type and number of spines of these two species. In the first there are two antapical spines and two or three accessory spines, but in *G. catenella* there are only two very short, hollow, hornlike, antapical spines. The delicate fin described as lying along the left margin of the ventral area of *G. catenata* is not found in *G. catenella*.

The cell contents of both species are much the same except for the nucleus. In *Gonyaulax catenata* this body is large and constricted, and in *Gonyaulax catenella* it is large, uniform in thickness, and U-shaped.

The peculiar type of chain formation in *Gonyaulax series* (Kofoid and Rigden, 1912) makes a comprehensive comparison of this species with *Gonyaulax catenella* impossible. Of course, the method of chain formation is a most striking difference, and for that reason a few comparisons may be noted. Instead of a thin strand of protoplasm connecting the members of a chain as seen in *G. catenella*, there was almost no constriction at all in the one chain observed in *G. series*. In this species the different individuals were fastened together in such a way as to obliterate the apical plates, parts of the postcingulars, ventral area, and antapical plate. The individuals in the chain varied in size, the largest being in the center and the others tapering in size toward the ends of the chain. The three individuals in the center were oriented alike, but the others may be turned to another angle. In *G. catenella* each individual of a chain is of the same size as the one adjoining it and they are always oriented in the same plane. The center individual of a *G. series* chain is about the same size as a single *G. catenella*.

Precingular plate 6'' in both species is quadrangular, but in *Gonyaulax series* it was proportionately much smaller. Postcingular plate 1''' is about the same size and similarly situated in both species.

Gonyaulax series, like *Gonyaulax catenella*, has a smooth, nonporulated surface with very faint suture lines. Thecal formation was not equally developed in all parts of the chain of *G. series* and may not be demonstrable in the more distal members. In *G. catenella* each member of the chain is equally developed, as much so as a single individual. Isolated individuals of *G. series* are unknown. Antapical spines are not present on any of the individuals of a chain of *G. series*, but may be in *G. catenella*.



The cell contents of these two species are densely granular and contain starch. In view of the lack of conclusive facts concerning the structure of the nucleus of *Gonyaulax series*, a comparison of this structure in the two species is omitted.

Gonyaulax acatenella, sp. nov.

Diagnosis.—A small rotund species resembling *Gonyaulax tamarensis*; plate formula is 4', Oa, 6'', 6, 6''', 1^p, 1''', surface smooth without pores or lists; theca free from markings; two short antapical spines and no apical horn; length 36 to 44 (39) μ , transdiameter 38–45 (40) μ ; coastal waters near San Francisco.

Description.—The body is rounded, with a tendency to be slightly wider than long in many individuals. The length is 0.92–1.02 of the transdiameter, dorsoventral diameter is 0.82–0.93 of the transdiameter. The *epitheca* exceeds the *hypotheca*. The *epitheca* is low and cone-shaped with convex sides. There is no apical horn. The *hypotheca* is broadly and symmetrically rounded.

The *girdle* (*gir.*, fig. 8) is postmedian, descending, displaced about 1 girdle width, without overhang, and deeply impressed without lists. The *longitudinal furrow* is deeply impressed with salient ridges and no lists. The *ventral area* widens greatly posteriorly and anteriorly indents the *hypotheca* slightly. The furrow is deeply impressed posteriorly and flares to the right. The middle region of the furrow measures at least 0.4 of the transdiameter. The posterior plate widens to about 3 girdle widths.

The plate formula is 4', Oa, 6'', 6, 6''', 1^p, 1'''. *Apical 1'* (fig. 8) measures about 1.3 girdle widths at its widest point. The side bounding on apical 4' is concave. *Apical 2'* (fig. 10) is elongated in the transverse direction and measures from 3 to 3.5 girdle widths in length. *Apical 3'* (fig. 10) is elongated in the longitudinal direction and measures from 2.5 to 3 girdle widths in length. *Apical 4'* (fig. 10) is nearly square; it is only slightly elongated in the transverse direction. It measures from 2 to 2.5 girdle widths in length. *Precingular 6''* (fig. 8) is quadrangular. *Postcingular 1'''* (figs. 8, 11) is small, rectangular, and very inconspicuous. It lies mostly in the longitudinal furrow. The ventral area proper consists of four intermediate plates. There are no lists on the margins of the ventral area. The *posterior intercalary* plate is elongated in the longitudinal direction and usually is wider at one end than at the other. At the widest point it measures approximately a girdle width.

EXPLANATION OF FIGURES

Gonyaulax acatenella

- Fig. 8. Ventral view of theca showing plates. $\times 840$.
 Fig. 9. Dorsal view of same. $\times 840$.
 Fig. 10. Apical view of *epitheca* showing plates. $\times 840$.
 Fig. 11. Antapical view of *hypotheca* showing plates. $\times 840$.
 Fig. 12. Ventral view of theca showing shape and position of the nucleus. $\times 840$.
 Fig. 13. Antapical view of same. $\times 840$.

Gonyaulax catenella

- Fig. 14. Midventral region of theca showing plates of ventral area. $\times 1700$.

Gonyaulax acatenella

- Fig. 15. Midventral region of theca showing plates of ventral area. $\times 1700$.

ABBREVIATIONS

1–6—girdle plates.
 1'–6'—apical plates.
 1''–6''—precingular plates.
 1'''–6'''—postcingular plates.
 1''''—antapical plate.
 gir.—girdle.

1^p—posterior intercalary.
 ant. pl.—anterior plate of ventral area.
 ap. po.—apical pore.
 a. sp.—antapical spine.
 cl. pl.—closing platelet.

fl. po.—flagellar pore.
 int. pls.—intermediate plates of ventral area.
 post. pl.—posterior plate of ventral area.
 v. a.—ventral area.

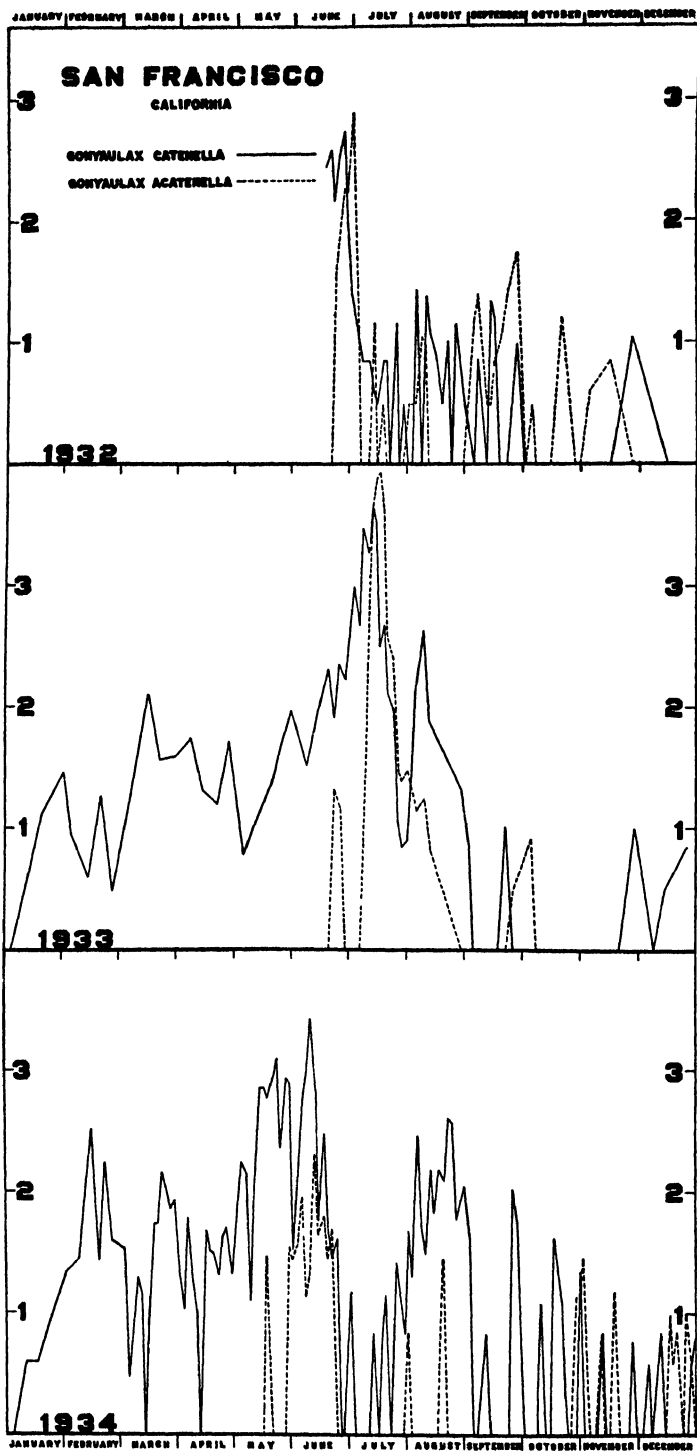


Fig. 16. Showing total numbers of *Gonyaulax catenella* and *Gonyaulax acatenella* during three successive years at San Francisco, California (logarithms of actual numbers per liter).

Two short antapical spines are always present. They are without lists and arise, one from the furrow side of the posterior intercalary plate near the antapex, and the other from the left antapex of postcingular plate 5'''.

The surface is smooth, nonporulated, and the suture lines are faint. In the closing plate (*cl. pl.*, fig. 10) a large pore is present which functions as an *attachment pore* (*ap. po.*, fig. 10). None of the individuals studied demonstrated the presence of a posterior attachment pore. Pores in the girdle plates, or plates adjoining the girdle region, are lacking. Lists are not present along the borders of the ventral area nor on the antapical spines.

Remarks.—In this species reproduction is known to be accomplished by skeletal fission. Apical plates 1' to 4', precingulars 1'' and 2'', girdle plates 1 to 3, and postcingulars 1''' to 4''' form the anterior moiety. Precingulars 3'' to 6'', girdle plates 4 to 6, postcingulars 5''' and 6''', the posterior intercalary plate 1^p, and antapical plate 1'''' form the posterior moiety. The fission line apparently passes through the ventral area in such a way that the anterior plate and flagella pore (*fl. po.*, fig. 15) pass to the anterior moiety, and the rest of the area, the intermediate plates, to the posterior moiety. No evidence of chain formation was found except once, when two individuals were seen attached in the usual manner.

The cell contents are not dense and are colorless to greenish. Examination under high magnification indicates that the cytoplasm is a reticulate or spongy mass. The nucleus is ellipsoidal and lies in the equatorial plane (figs. 12, 13). There are few starch granules, few chromatophores, and few oil droplets. The last-named, when present, lie near the nucleus.

Dimensions.—Sixteen individuals were measured. Length (exclusive of spines) 36–44 (39) μ ; transdiameter 38–45 (40) μ ; dorsoventral diameter 32–41 (36) μ ; width of the girdle 5–7 (6) μ ; length of the antapical spines 2 to 4 μ . Most individuals, as the measurements indicate, were slightly wider than long, but about one-fourth of the number were of the reverse proportions.

Variation.—Varies only slightly in size and proportions. There may be some variations in the color, but this is usually rather slight.

Distribution.—This species has been collected in Pacific waters in the vicinity of San Francisco and Crescent City, California, and Brookings, Port Orford, Bandon, and North Bend (Coos Bay), Oregon. It is a neritic species and most abundant in waters of approximately 15° C in temperature. In the summer of 1932, *Gonyaulax acatenella* first appeared in the collections on June 24 and remained for about a week, appearing again in small numbers intermittently in August, September, and October. This species appeared on June 23, in 1933, and remained until August 12. In 1934 this form was present intermittently from May 18 to August 22. It has always appeared at approximately the same time as *G. catenella* (fig. 16).

Comparisons.—This species and *Gonyaulax tamarensis*, described by Miss Lebour (1925), are very closely related. She has kindly checked our species, so that we are able to state here the following conclusions. *G. tamarensis* is found only in estuarine waters, whereas *G. acatenella* has been found only in waters of the open sea. In size, shape, arrangement of the plates, and plate formula, the two forms are very similar. The major difference between the two

species lies in the shapes of the apical plates. Apical 1' of *G. tamarensis* is quite broad and very characteristically shaped. The same plate in *G. acatenella* is much more slender and regularly shaped. Apical 2' of this same species is pentagonal, approximately as wide as it is long, but in *G. tamarensis* the same plate is distinctly rectangular. Apical 3' in the first species is larger than apical 2' and pentagonal. In the Tamar species this plate is also pentagonal, but it is smaller than apical 2'. Apical 4' in both species is a large irregularly shaped plate, only slightly elongated in the transverse direction in *G. tamarensis*, and greatly elongated in that direction in *G. acatenella*. In the former, the width of the plate in the drawings is nearly the same at both ends, whereas in the latter, the end nearest to precingular 3'' is very narrow and the opposite one very wide. Minor differences exist in that the posterior intercalary plate in *G. acatenella* is smaller in proportion to the antapical plate than it is in *G. tamarensis*, but is very similarly shaped, and the antapical plate is hexagonal whereas in the latter species it is pentagonal and proportionately larger than in *G. acatenella*.

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**SPAWNING HABITS OF THE MUSSEL
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**WITH NOTES ON THE POSSIBLE RELATION
TO MUSSEL POISON**

MUSSEL POISON. I

BY

W. FOREST WHEDON

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MUSSEL POISON. I

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(Contribution from the George Williams Hooper Foundation for Medical Research, University of California, San Francisco)

EARLY INVESTIGATION of mussel poison indicated that its nature was possibly much like that of Fugu poison, found in the gonads and digestive diverticula of toad fishes. According to Meyer, Sommer, and Schoenholz (1928), there was some suspicion that mussel poison might also be the basic product of the sex metabolism of the mussel. They suggested that this assumption did not explain the sudden appearance of the highly potent toxin at regular intervals. In the years which followed, however, changes in the appearance of the digestive diverticula were observed, which led to the belief that a thorough study of the spawning habits of *Mytilus californianus* in relation to the poison was of importance.

Stating that a definite spawning period was unknown, Rankin (1918) reported that dealers in shellfish had suggested that the mussels were spawning as early as April or May, but pointed out that the mussels in the region around San Diego were not in a spawning condition until late July. Working at Scripps Institution of Oceanography, La Jolla, California, Coe (1932) showed that attachment of *Mytilus californianus* to experimental blocks takes place as late as October, and that free-swimming larvae are very numerous in the warmer summer months. This would indicate that the mussels spawn from June to September.

Of importance is the size and age of the mussels, as the results of some earlier investigations upon *Mytilus edulis* L. have also shown. Wilson (1887), McIntosh (1885), and Williamson (1907) reported that mussels varying in size from three-eighths of an inch to $1\frac{3}{4}$ inches had been observed to spawn. With the exception of Scott (1901), investigators agree that fertilization takes place outside of the body. *Mytilus californianus* were found to be in a spawning condition when the individuals measured but an inch in length. This finding was usually true of the males. Females measuring $1\frac{1}{2}$ inches in length have been found in a spawning condition. While the exact age of this size of mussel cannot be definitely stated, it is probably about one year.

Another account of spawning of Pacific Coast mussels is one by R. Stohler (1930), who worked on *Mytilus californianus* and collected his material at

Mussel Rock. He has reported only nonspawning ("empty") mussels from March 7 to June 13, 1929; but from June 25 until August 1, of the same year, all mussels examined were full of eggs or sperm. By the term "empty," he infers that the mussels contained no macroscopically visible ripe or ripening sex products. On August 5, the spawning period was over, and all mussels were empty from that date until November 4, 1929, when the formation of germ cells became very pronounced. From November 18 to January 14, 1930, they were again full of spawn. Then from January 27 to March 12, when his observations ended, he found only "empty" mussels.

H. Sommer has kindly offered the use of unpublished data concerning spawning in mussels which he has examined and used for chemical tests. He found that, during the summers of 1928 and 1929, at Mussel Rock, the mussels were usually full of spawn. In August, 1928, at the same point, some of the animals had discharged; but as late as September 2, 1929, he found no "empty" mussels. In 1933, while at La Jolla, California, he examined mussels, on May 17 and May 26, measuring from 2 to 3 inches in length. These were found to be about spawned out.

The present observations, with additional periodic examinations, were conducted between October, 1931, and October, 1932. Material for study was collected from Pescadero, Half Moon Bay, Mussel Rock, Pedro Point, San Francisco, Bodega, and Jenner. These places named in order from south to north, cover a distance of one hundred and fifty miles. Only those individuals from which ova and spermatozoa flowed, after the mantle and mesosoma had been punctured, were considered as mature and in a spawning condition. Each determination was made with use of at least fifty mussels, averaging $3\frac{1}{2}$ inches in length.

Reference to the accompanying graph shows that the maximum spawning in 1931 occurred about November 6, when 90 per cent was recorded. There is one period, from October 25 to November 23, 1931, when at least 80 per cent of all individuals examined were spawning. From this date until January 20, 1932, the percentages dropped to approximately 40 per cent and rose again to 72 per cent, after which there was a gradual decline followed by another small increase in May and June. Beginning August 1, there was a gradual increase in the percentage of mature individuals.

On June 20, the difference in percentage of males and females was most pronounced; 64 per cent of the females were mature, whereas only 28 per cent of the males were in a spawning condition. Variations in the maturing of the two sexes have also been described by Williamson (1907) for *Mytilus edulis* and *Modiolus modiolus*.

If the percentages of spawning mussels for October 28, 1931, and October 28, 1932, be compared, it will be seen that there is a difference of approximately 35 per cent. On November 2, 1933, 67 per cent of the mussels were recorded as mature, and on the same date in 1931 88 per cent were in the same state. Thus we find that the ratios of spawning vary considerably, but do fall within approximately the same time limits. The observations of Dr. Sommer

for 1928 and 1929, while made on small collections only, tend to substantiate these findings. There are then, based on figures for 1931-1932, a period of maximum spawning, two "secondary" periods, and for some mussels, spawning throughout the whole year.

In order to determine any possible variation in the spawning habits, mussels for this investigation were procured from various points along the coast in the region of San Francisco. Monthly collections of mussels, ranging in size from one or two millimeters to two centimeters, were made throughout the year. It was found that, no matter when or where the mussels were collected, there were always small mussels in the clumps of adults among the byssus threads. Plate 3 shows some of these collections.

Quite obviously, these observations and those by Stohler do not agree closely. One or two of his dates of observing "empty" mussels agree with the periods of low-percentage spawning shown here. However, the report is misleading because he has overlooked the approximate 20 per cent that were spawning, and failed to note the actual numbers of spawning individuals.

On the contrary, the present findings agree very closely, except for dates of spawning, with those of Johnstone (1899), working with *Mytilus edulis* in England, who states:

As a result of this year's observations, it has been found possible to fix approximately the date of a maximum in the spawning of the Mussel, during which a rapid and complete extrusion of the genital products, accompanied by other histological changes in the mantle and visceral mass of the animal, takes place. In the year 1898 this was found to begin about the beginning of July and last till about the beginning of August; but it is probable that the limits of this period are variable to some extent. There is, however, considerable doubt as to whether this is the only time in the year during which spawning takes place, and various observations render it at least possible that there is a secondary spawning period early in the year, and that there is a continual but slow emission of ova and spermatozoa from the time when these have accumulated in considerable quantity in the gonads, that is to say, from the beginning of April on to the beginning of the summer spawning period. And it seems certain, considering the variability observed in the ripening of the gonads, that isolated individuals may undergo complete spawning a considerable time in advance of, or later than, the date of occurrence of the maximum.

Many investigators have attempted to explain the factors that influence the time and act of spawning in mussels, but little correlation of fact is found in their reports. Finding a relationship between the spawning period of an echinoid (*Centrechinus setosus*), found in the Red Sea, and the lunar phenomena, Fox (1922) and his assistants made a similar study of *Mytilus edulis* in the same region, and at Southampton, England. They found no correlation of this nature where this form was concerned. Here on the Pacific Coast, no evidence of a like relationship could be found.

In discussing the reasons why *Mytilus galloprovincialis* had been reclassified as a variety of *Mytilus edulis*, Orton (1920) suggested that temperature conditions for reproduction and the necessity for adapting themselves to another environment had been reasons for the original classification. Mussels at Buddle Bay, Northumberland, spawned from July into September in 1907, but have been found to spawn earlier in warm seasons than in cold (William-

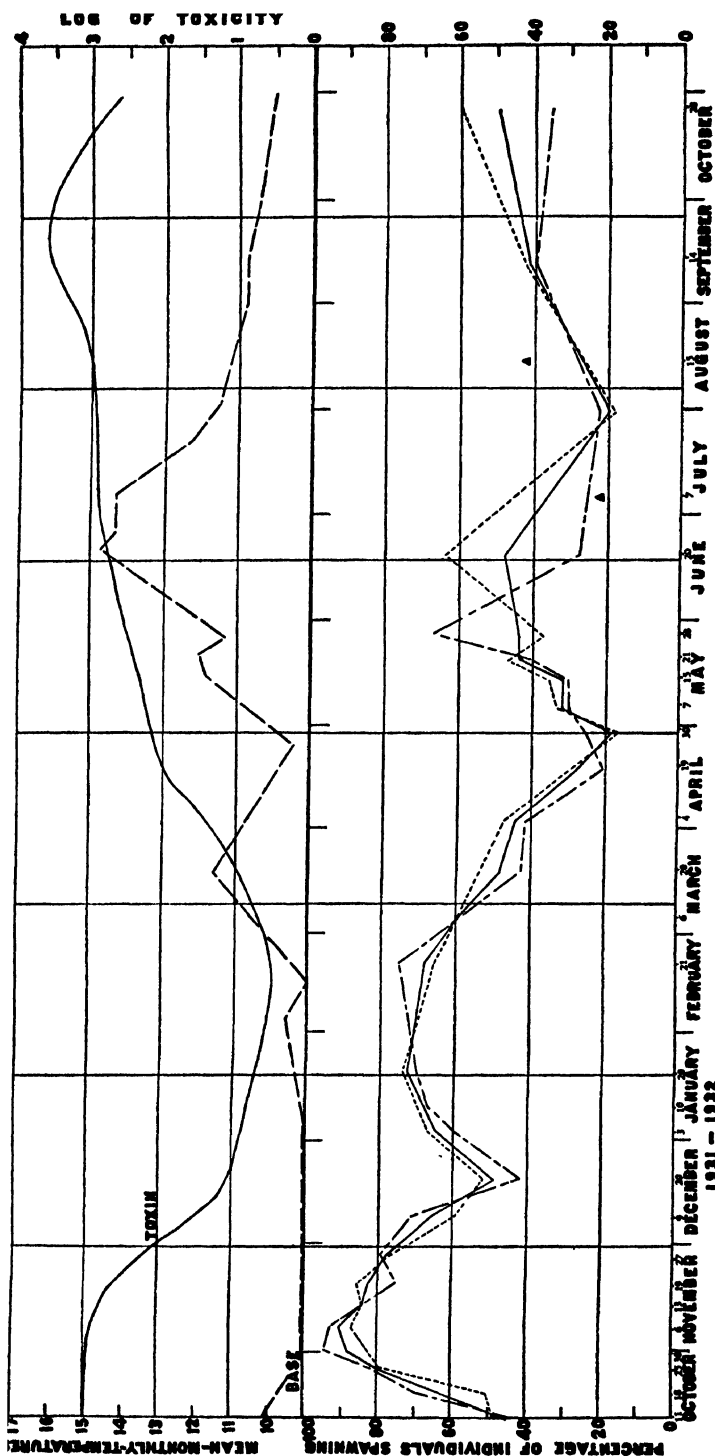


Fig. 1.—The relation of temperature and toxicity to the percentages of spawning mussels during 1931 and 1932 at San Francisco. Total percentage of males and females spawning is shown by a continuous line; percentage of females spawning, by an evenly broken line; percentage of males spawning, by an unevenly broken line; and total percentage of males and females at Bodega, California, by black pyramid.

son, 1907). Temperatures, both of water and of air, doubtless play a part in the rate of development of the spawn, but whether the temperature changes of the region near San Francisco are severe enough to cause spawning, is questionable. Water temperatures in the region of San Francisco have varied from approximately 10° C to as high as 17° C over a period of more than a year. The highest temperatures are usually found from July to October, and the coldest from December to February, the change from one period to another being very gradual. *Mytilus edulis*, on the Atlantic Coast, develops in the coldest months and begins to spawn with the onset of warmer conditions (Field, 1922). Spawning individuals were found only in the summer months. On the contrary, the findings for Pacific Coast mussels in the San Francisco region indicate that the sex products develop in the months when the water temperatures are highest and food is most abundant, and the mussels spawn in the cooler months. (See graph, p. 38.) The mean water temperature for the maximum spawning period of 1931 was 10° C. When mussels have been experimented with in the laboratory, they have spawned without any apparent reason, especially the males. Females that have spawned, spawned at night, but did not completely empty the sex organs. Keeping the mussels out of water for a period of twenty-four hours or longer at room temperature has, upon several occasions, apparently caused them to spawn. However, these findings are not conclusive evidence, and do not shed any further light on the stimuli which cause spawning.

Field (1922), Daniel (1922), and others have definitely shown that in *Mytilus edulis*, as the sex products develop, there is a corresponding change in the other organs of the body, and that this is especially true of the digestive diverticula. As the observations on *Mytilus edulis* correspond very closely with those of *Mytilus californianus*, further detailed discussion is unnecessary.

The color of the digestive gland is in large measure controlled by the various pigments, mostly green, which are found in the marine plankton used as food material. With the increase in sex products and the intrusion of these products among other tissues, the color of the digestive diverticula may become quite brown, depending upon the degree of development of the sex products. The gland in many poisonous mussels was observed to be enlarged and soft, and since earlier investigations of mussel poison in European countries found a similar condition, it was suspected that there might be some connection between the spawning period and the production of the toxin. According to Wolff (1886), it was commonly believed in Germany that mussel poison was closely allied to the development of the sex products, as the mussels were most poisonous in the hot summer months when the mussels were spawning. The findings for the San Francisco area show that the maximum poison may occur either at a time when very few mussels are spawning or when large numbers of them are spawning (see graph). The author is indebted to Dr. H. Sommer, who kindly permitted the use of toxin values from his manuscript, which is to be published soon.

CONCLUSIONS

There is evidence that for the Pacific Coast mussel, *Mytilus californianus*, in the San Francisco region, there is a maximum period of spawning beginning early in October, followed by two other periods of lesser degree, in January and February, and May and June. It has been shown that spawning occurs at all times of the year, apparently irrespective of temperature or other external stimuli.

No evidence was found that spawning of this mussel parallels either the increase or the decrease of mussel poison.

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EXPLANATION OF PLATE

Plate 3. The various stages in the growth of the mussel, *Mytilus californianus*, following the formation of the shell. Natural size. The collections were made at different places in California, as follows:

A. Half Moon Bay, October 11, 1931

B. Jenner, April 23, 1932

C. Pedro Point, April 28, 1932

D. Pescadero, June 18, 1932

E. San Francisco, September 14, 1932



A



B



C



D



E

**NEW SPECIES OF SPIONIDÆ
(ANNELIDA POLYCHAETA)
FROM THE COAST OF CALIFORNIA**

**BY
OLGA HARTMAN**

**UNIVERSITY OF CALIFORNIA PUBLICATIONS IN ZOÖLOGY
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NEW SPECIES OF SPIONIDAE (ANNELIDA) POLYCHAETA) FROM THE COAST OF CALIFORNIA

BY
OLGA HARTMAN

THE SPECIES of Spionidae herein described were collected by the author from the littoral of California between Fort Bragg, Mendocino County, and Pacific Grove, Monterey County, California, including the estuarine and brackish waters of San Francisco Bay and Lake Merritt, Oakland. More specific localities are given in the descriptions.

Holotypes are to be deposited in the United States National Museum, paratypes in the University of California and the California Academy of Sciences.

DESCRIPTIONS OF SPECIES

Streblospio luteicola sp. nov. (figs. 1 and 2)

Measurements.—8–12 mm. long, 0.6–1.5 mm. wide; number of segments about fifty.

Prostomium a rounded snout projecting forward and somewhat upward at its tip (fig. 1); provided with 4 reddish eyes disposed in a wide-open crescent between bases of palpi; palpi much as in the genus *Polydora*, pale, with an anteroventral, ciliated, longitudinal groove, in life generally projecting forward and hidden by the overlying peristomial cirri.

Peristomium a ventral prolongation forming the lower lip (= ventral collar of Buchanan), present dorsally only as a point of attachment for the peristomial tentacles; passing imperceptibly into second segment (fig. 1).

Peristomial tentacles a pair of dorsoventrally flattened, respiratory organs originating from the dorsalmost part of the peristomium, slightly behind region of eyes (fig. 1), with sinuous margins and slender apex, crossed by 4–7 dark or ashy green bands of pigment at irregular intervals.

Dorsal collar on segment 3 (fig. 1), formed by an expansion and fusion of the dorsal post-setal lamellae; with a conspicuous, median, glandular area on the dorsum behind the collar.

Capillary setae in tiny, dorsal fascicles of 3–5, and in ventral fascicles of 4–6 in segment 2, increasing to 12–15 setae in a dorsal fascicle in segment 3 and continuing so through six segments; capillary setae present in notopodia throughout, diminishing in numbers in posterior neuropodia, where they are accompanied by crotchets.

Hooded crotchets with 4 distal teeth (fig. 2), first present in neuropodia of segment 8 in which there are 4 crotchets accompanied by an inferior fascicle of a few capillary setae; more posteriorly the number of crotchets increases and that of capillary setae decreases, the ten most posterior segments with 8–10 crotchets and a single inferior capillary seta.

Anus terminal, bordered by an inconspicuous collar which is only slightly wider than the last segment.

Tube weakly chitinized inside, covered with particles of soft debris; often five or six times as long as worm; disposed obliquely to upright in the mud, with the head end projecting slightly above the level of the substratum.

Color pale greenish gray or almost colorless; peristomial cirri with irregular rows of grayish green on the lateral flanges; eyes reddish.

Distribution and biology.—This species belongs to a genus to which only three species have been attributed, *S. benedicti* Webster from the northeastern

coast of the United States, *S. shrubsolii* (Buchanan) from estuarine waters of northwestern Europe, and *S. dekjuyzeni* Horst from the Zuiderzee, the last-named being probably identical with *S. shrubsolii* (Buchanan). *S. lutincola* inhabits brackish waters or at least waters of lowered salinities. It is abundant in the mud flats of east San Francisco Bay, in the muddy bottom of Lake Merritt, Oakland, in the southern end of Tomales Bay, near Inverness, and in Bodega Lagoon, north of Bodega Bay, California.

Feeding habits of *S. lutincola* are much like those of *Boccardia natrix* (Söderström): relatively large food masses are nipped off by the distal ends of the palpi, taken into the ciliated palpal groove, and carried into the ventral mouth. Fecal particles are long, cylindrical, and ejected from the anterior end of the tube on the ventral side.

Systematic discussion.—*Streblospio lutincola* differs from *S. shrubsolii* (Buchanan) in that (1) the dorsal collar is a single piece, (2) the peristomial tentacles are foliaceous, (3) color in life is pale greenish. *S. lutincola* has been compared with individuals of *S. benedicti* Webster from New York harbor. From this species it differs conspicuously in its much smaller ventral peristomial fold, and its much larger prostomium.

EXPLANATION OF FIGURES

(All figures were drawn with the aid of a camera lucida)

Figs. 1, 2. *Streblospio lutincola*

Fig. 1. Anterior end from right side with right palpus and peristomial cirrus removed, showing ventral fold under prostomium and dorsal collar. ($\times 14$)

Fig. 2. Hooded crotchet from a posterior parapodium. ($\times 492$)

Figs. 3-5. *Polydora brachycephala*

Fig. 3. Anterior end in dorsal view, palpi and setae not shown. ($\times 14$)

Fig. 4. Hooded crotchet from a posterior neuropodium. ($\times 222$)

Fig. 5. Special seta from sixth segment. ($\times 222$)

Figs. 6-10. *Polydora amarincola*

Fig. 6. Anterior end through sixth segment in dorsal view, left palpus and setae not shown. ($\times 14$)

Fig. 7. Hooded crotchet from a posterior neuropodium. ($\times 492$)

Fig. 8. Special seta from sixth segment in worn condition. ($\times 222$)

Fig. 9. Special seta from sixth segment, unworn. ($\times 222$)

Fig. 10. Anal end in dorsolateral view, showing anal disk with dorsal notch. ($\times 37$)

Figs. 11-16. *Boccardia truncata*

Fig. 11. Anterior end in dorsal view, with palpi removed. ($\times 14$)

Fig. 12. Anterior end in ventral view, setae not shown. ($\times 14$)

Fig. 13. Hooded crotchet from a posterior neuropodium. ($\times 222$)

Fig. 14. Special seta from sixth segment. ($\times 222$)

Fig. 15. Pennoned companion seta from sixth segment. ($\times 222$)

Fig. 16. Anal disk from posterior surface. ($\times 14$)

Figs. 17-19. *Pygospio californica*

Fig. 17. Anterior end in dorsal view, showing tentacles on third segment, most of left palpus not shown. ($\times 37$)

Fig. 18. Hooded crotchet from a posterior neuropodium. ($\times 492$)

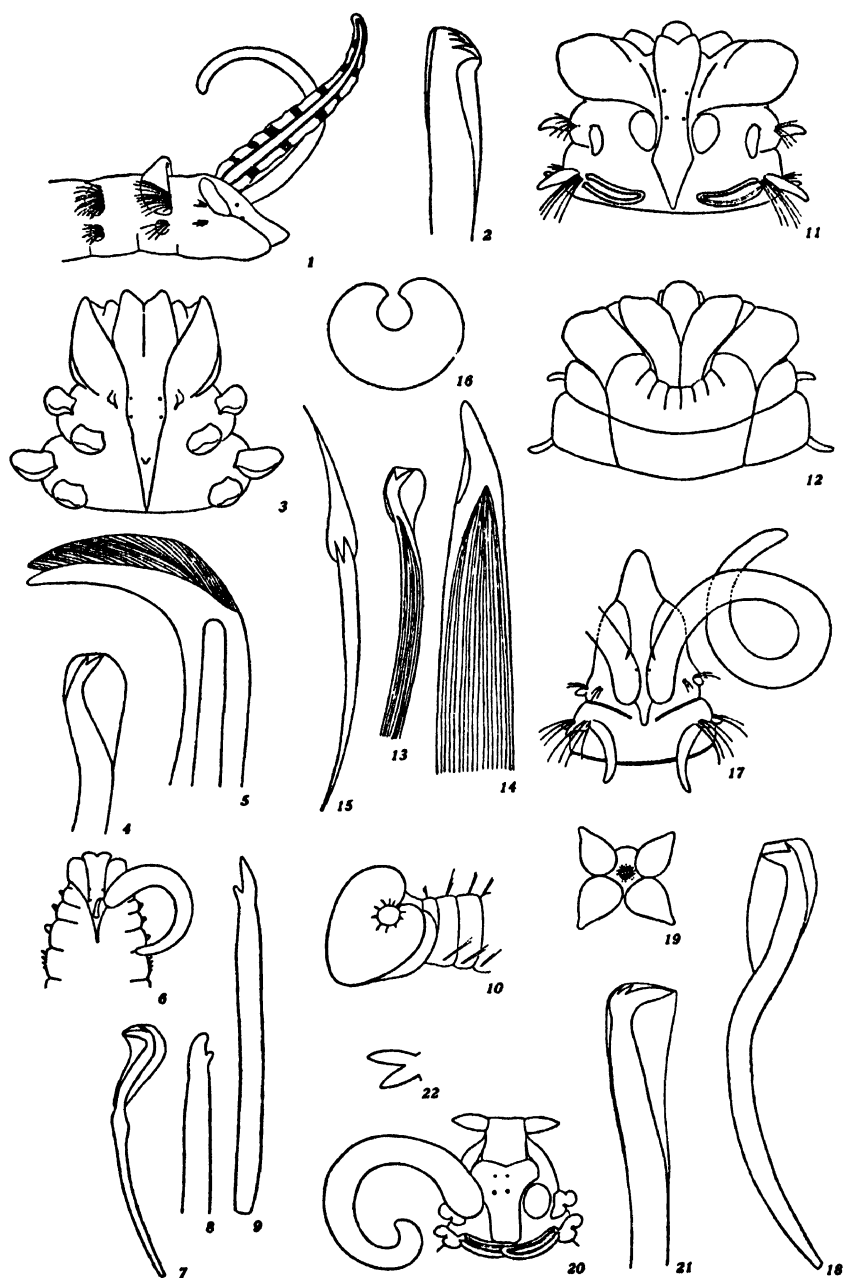
Fig. 19. Anal cirrus in posterior view. ($\times 37$)

Figs. 20-22. *Rhynchospio arenicola*

Fig. 20. Anterior end in dorsal view, right palpus and setae not shown. ($\times 37$)

Fig. 21. Hooded crotchet from a posterior neuropodium. ($\times 492$)

Fig. 22. An anal, bilobed cirrus. ($\times 53$)



Polydora brachycephala sp. nov.

(figs. 3-5)

Measurements.—Length 25–40 mm.; width at sixth segment about 2.2 mm.; number of segments about 100.

Prostomium a fleshy prolongation (fig. 3), dorsally convex, produced anteriorly to form a deeply incised, slightly tapering lobe, in line with the broad, deep, buccal pouches (fig. 3); depressed in region of the eyes and tapering posteriorly into a low caruncle which extends through the second setigerous segment; most individuals with a low, hardly discernible, nuchal tentacle present near tapering end; prostomial ridge with 2 pairs of minute, black eyespecks between bases of palpi; palpi stout, long, arising from an area directly in front of first dorsal lamellae, as long as first twelve segments, deeply grooved on anterior border, the folds at sides of groove marked by a conspicuous, purplish brown pigment.

Peristomium forming laterally a pair of buccal pouches, and ventrally a broad lower lip which is marked with 9 or 10 shallow, longitudinal grooves, extending posteriorly in median region so as to cut into second and third segments.

Branchiae from eighth segment, fused to dorsal lamellae on their proximal borders; branchiae of opposite sides hardly meeting in middorsal region; longest in anterior half of body; absent from 15–20 posterior segments.

Parapodia with conspicuous dorsal and ventral postsetal lamellae on segments 2–5 (fig. 3), becoming gradually smaller on segments more posterior; with a few capillary setae in notopodia and neuropodia of second segment (first setigerous), in double series in all other notopodia but segment 6, and in neuropodia of segments 3–5, and 7; neuropodia of segment 8 with nine hooded crotchets (fig. 4) accompanied by an inferior fascicle of a few capillary setae, this arrangement continuing posteriorly except with a gradual decrease in number of setae.

Modified sixth segment with each notopodium provided with a tiny superior fascicle of capillary setae and 10–12 stout, amber-colored, special hooks having a posteriorly projecting spine and an outer, subterminal covering of fine, long hairs (fig. 5); each neuropodium with about 15 capillary setae and a greatly reduced postsetal lamella.

Anal disk a thickened collar, broadest ventrally, with a dorsal notch.

Distribution and biology.—*Polydora brachycephala* is abundant in the mud flats of Drake's Estrero, Marin County, and in the sandy mud flats of the southern end of Bodega Lagoon, Sonoma County, where it is found with a cirratulid, just below the extensive sand flats which harbor *Boccardia natrix* (Söderström) (= *B. californica* Treadwell). Interestingly, it is also present about one mile inland from the mouth of Stempell Creek, Marin County, a tidal stream with varying salinity, where the worms construct deep, vertical burrows, extending through a thick layer of 6–8 inches of sandy clay to a very hard clay substrate which the worms penetrate with difficulty. Here the only polychaetous associate is a species of *Neanthes* (to be described in a later paper) which likewise burrows in the hard-packed soil. The collections of the University of California contain two individuals labeled "*Polydora anoculata*" and "*Polydora californica*," both of which are *P. brachycephala*. These were collected in San Francisco Bay, on the north side of the Golden Gate.

Polydora brachycephala is readily distinguished in the field from *Boccardia natrix* (Söderström), with which it is often associated, by its larger, more robust appearance, by the absence of branchiae anterior to the modified sixth segment, and by its broad, truncate prostomium.

Systematic discussion.—The special hooks of the sixth segment place *B. brachycephala* closest to *P. caulleryi* Mesnil, from western Europe. *P. brachycephala* is distinguished by having dorsal setae in the first setigerous segment, and in having eyespots.

Polydora amarincola sp. nov.

(figs. 6–10)

Measurements.—Length 3–10 mm.; width less than 1 mm.; number of segments 40–55.

Prostomium bifid in front (fig. 6), with 2 pairs of black eyespots between bases of palpi; with a median tentacle (fig. 6) on the prostomial elongation posterior to eyes; palpi grooved on anterior side, attached just dorsal to first neuropodium.

Peristomium consisting of a pair of fleshy, lateral pouches at sides of prostomium, longer than wide (fig. 6); produced ventrally to form a smooth, lower lip.

Branchiae from eighth segment, absent from last 8 or 10 segments; curved over dorsum; branchiae of eighth segment about two-thirds as large as those more posterior.

Capillary setae in all notopodia except second and sixth segments; present also in neuropodia of segments 2–5, in segment 7 in a double series, and in the inferiormost part of more posterior somites as a tiny fascicle of a few setae ventral to the uncini.

Hooded crotchets (fig. 7) in single series from eighth segment, at first with about 4 crotchets in a fascicle, increasing to 7 in the ninth segment, and decreasing gradually in number in posterior region to 2 crotchets in the last fascicle.

Modified sixth segment with 5–8 large hooks in a single series, worn hooks in superior part of fascicle, bluntly rounded and with a conspicuous subterminal tooth (fig. 8), inferior hooks less worn or immature, with a conical tip (fig. 9).

Anus surrounded by a disk with dorsal notch (fig. 10), about twice as wide as last setigerous segment.

Tube covered externally with débris, internally more or less chitinated; 1 to 3 inches long; egg masses lining inner, lower half of some tubes.

Distribution and biology.—*Polydora amarincola* is abundant in and under colonies of *Mercierella enigmatica* Fauvel in the brackish waters of Lake Merritt, Oakland, California. It is present also in decayed piles, its tubes occupying crevices made by fouling organisms, and in the muddy bottoms of Lake Merritt, the Oakland estuary, and San Francisco Bay, eastern side. One-eighth pint of mud from a Berkeley beach contained 52 tubes, of which about half appeared to be deserted. It has been taken also from under *Balanus* beds at Moss Beach, San Mateo County, California.

Systematic discussion.—*Polydora amarincola* is separable from *P. giardi* Mesnil—which has been collected by the author at Fort Bragg, Mendocino County, California—by the presence of a median, nuchal tentacle, and by the structure of the stout hooks of the sixth segment, which are almost straight in *P. amarincola*. It is the smallest of the known species of *Polydora* (s. lat.) from California.

Polydora truncata sp. nov.

(figs. 11–16)

Measurements.—Length 8–20 mm.; width at sixth segment 1 mm.; number of segments 40–60, rarely 100; a long, slender species.

Prostomium a low ridge, widest anterior to eyes, slightly incised at its anterior margin (fig. 11), extending posteriorly beyond palpal bases to form a slightly widened area which tapers to a point in the region of the first branchial segment (fig. 11); with 4 tiny, black

eyespots disposed in rectangular arrangement between bases of palpi; with a pair of grooved palpi which are stout at base and taper to blunt tips.

Peristomium with a pair of prominently distended, broadly truncate, buccal pouches which extend dorsolaterally (fig. 11), forming ventrally a concave lower lip marked by several longitudinal grooves (fig. 12), marked off from second segment by a shallow, transverse groove.

Branchiae normal on segments 3 and 4, absent from 5 and 6, tiny on 7, larger from eighth segment to middle region of body; never touching across dorsum; absent from posterior fifth of body; entirely free from dorsal lamellae.

Parapodia with cirriform postsetal lamellae, tiny on segment 2, longest on segments 3-5, becoming successively smaller from segment 7; ventral lamellae similar to dorsal lamellae but only about two-thirds as large; all notopodia with capillary setae except first setigerous segment, present in two series except in sixth segment; neuropodia with only capillary setae in segments 2-7, and with capillary setae and hooded crotchets (fig. 13) in segments from eighth, the capillary setae restricted to inferiormost part of neuropodia and not present in the last few neuropodia.

Modified sixth segment with setae of three kinds, capillary setae in neuropodium and in superior part of notopodium above the special hooks; 7-10 special hooks (fig. 14) with a blunt point and a subapical concavity on posterior side, accompanied by pennoned setae (fig. 15).

Anus surrounded by a wide collar open dorsally (fig. 16), with a flange thickened centrally but tapering to a thin, free edge.

Color.—Greenish brown in life, lacking sooty, prostomial pigmentation characteristic of *Boccardia natrix* (Söderström), though often with sooty spots behind bases of parapodia; body fluid deep brown.

Locality.—Known only from type locality, about one-fourth mile south of Moss Beach, San Mateo County, California, from soft sandstone reefs at low-tide level.

Systematic discussion.—*Boccardia truncata* approaches *B. redeki* Horst from Holland more nearly than any known species. It differs from this species in the structure of its modified hooks and in having a much broader, shorter "head."

Pygospio californica sp. nov.
(figs. 17-19)

Measurements.—Length 10-15 mm.; width at tenth segment 0.6 mm.; number of segments about 85.

Prostomium with a median, anterior prolongation ending in a blunt cone (fig. 17); extending posteriorly as a low caruncle through the first setigerous segment (= segment 2); provided with 2 pairs of eyes, the anterior pair elongated, situated in the groove between prostomium and peristomium, the posterior pair rounded, nearer together, concealed by the palpi; palpi massive, tapering, palpal groove bordered by a line of dark pigment.

Peristomium forming a pair of long, lateral, slightly inflated pouches (fig. 17), indistinctly marked off from prostomium; extending ventrally to form a conspicuous lower lip that projects posteriorly as far as segment 3.

Branchiae from nineteenth segment to posterior fourth of body; of the 35-45 pairs present the first 10 pairs relatively large, almost meeting across the dorsum, the next 25-35 gradually decreasing in size, the last 10 becoming mere stubs; branchiae heavily ciliated on outer margins; fused to dorsal lamellae on their proximal edges.

Parapodia anteriorly with conspicuous dorsal and ventral postsetal lamellae which extend distally to middle of setae; second setigerous segment with a pair of dorsal, cirriform tentacles exceeding the second segment in length (fig. 17); hooded crotchets first present in neuropodia of twenty-third setigerous segment, provided with 2 terminal teeth (fig. 18); notopodia with capillary setae only.

Anus surrounded by 4 conical papillae of which the ventral pair is the larger; dorsal pair smaller and separated (fig. 19).

Locality.—Known only from type locality, in eel grass and sand flats at medium low-tide level, near Princeton, San Mateo County, California.

Distribution and systematic discussion.—This is the first record for the genus *Pygospio* from the North Pacific and possibly for the entire Pacific. Monro, in 1930, described *Pygospio dubia* from South Georgia but says that it "may be the young of a *Polydora*." There is only one well-known species, *P. elegans* Claparède, from the Atlantic, and most authors have placed all other described species into synonymy with this. From *P. elegans* Claparède, *P. californica* is clearly distinguishable in the shape of its prostomium and in the distribution of its branchiae.

Rhynchospio gen. nov.

Description.—Prostomium with frontal horns, with a pair of long, grooved palpi; branchiae from second setigerous segment (= segment 3) and present on most segments; notopodia with capillary setae only; neuropodia with capillary setae and hooded crotchets; anus surrounded by cirri; differing from *Scolecopsis* Blainville in lacking branchiae on first setigerous segment.

Type: *R. arenicola* sp. nov.; *type locality*: Moss Beach, San Mateo County, California.

Rhynchospio arenicola sp. nov.

(figs. 20–22)

Measurements.—Length 7–10 mm.; width at tenth segment 0.6 mm.; number of segments about 45.

Prostomium with 2 frontal horns (fig. 20) which project laterally; with an almost straight, anterior border; a trapezoidal anterior portion set off from a broader posterior portion by a transverse notched line (fig. 20); produced posteriorly to form a low, truncate keel; provided with 4 round, black eyespecks, the anterior pair minute, the posterior pair larger, disposed between the bases of the palpi; palpi 2, robust, with an anterior groove.

Peristomium with a pair of lateral pouches (fig. 20), longer than wide, extending ventrally to form a smooth, lower lip which tapers posteriorly and passes imperceptibly into a ventral, longitudinal stripe bordered by two white lines.

Proboscis a soft, protrusible pouch, capable of extension to a volume equaling that of prostomium and peristomium.

Branchiae from third segment (= second setigerous) to posterior end; straplike, held over dorsum, those in anterior region partly overlapping (fig. 20); becoming gradually tiny in posterior part; almost free from dorsal lamellae.

Paradodia with foliaceous postsetal lamellae in anterior region, becoming smaller posteriorly; with dorsal glands from seventh segment; hooded crotchets first present in eighteenth segment in a single series, tridentate apically (fig. 21), accompanied by a few inferior capillary setae to the posterior end.

Anus surrounded by 2 pairs of bilobed cirri on each side, the lobes deeply incised (fig. 22), giving the impression of 8 distinct cirri.

Locality.—In low-tide, shallow, sandy pools, near Moss Beach, San Mateo County, California; known only from type locality.

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**SOME NEW AND LITTLE KNOWN
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INTRODUCTION

THE AMPHIPODA described in the following pages represent part of the results of intensive collecting over a small area in the vicinity of Moss Beach, San Mateo County, California, during June, July, and August of 1933. The Amphipod fauna of this region proved to be quite extensive. Consequently the members of the families Calliopidae, Pontogeniidae, and Talitridae, and all that were of the tribe Caprellidea were put aside for future study. Moreover, three species of the family Ampithoidae, present in the collection, remain undescribed, since only females were collected.

Works on the Amphipoda of the Pacific Coast of North America are relatively few; hence it has been thought worth while to enter all those that were encountered in the bibliography. Such references will be found marked with an asterisk (*).

I wish to express my gratitude to Professor S. F. Light, of the Department of Zoölogy, University of California, both for the original impetus which resulted in these studies and for later aid in the formulation of the descriptions.

METHODS OF COLLECTING

Most of the forms described were taken from rocks lying in about two fathoms of water, to which the holdfasts of *Neriocystis Luetkeana* were attached. These were pulled up by means of the attached algae, and the animals removed with forceps and pipette in the laboratory.

Certain of the night-swimming pelagic forms, especially *Nototropis* and some of the Pontogeneids, were taken by means of an electric light trap. A few of the described forms were collected by the usual methods of turning over rocks, washing out the fronds of algae, etc.

SYSTEMATIC LIST OF SPECIES DISCUSSED

Family Phoxocephalidae

Pontharpinia obtusidens sp. nov.

Family Pleustidae

Pleustes depressus sp. nov.

Family Atylidae

Nototropis tridens sp. nov.

Family Gammaridae

Maera inaequipes (A. Costa)

Elasmopus brasiliensis (Dana)

Melita californica sp. nov.

Family Dexaminidae

Polycheria antarctica (Stebbing)

Family Aoridae

Aoroides californica sp. nov.

Family Photidae

Photis conchicola sp. nov.

P. reinhardi Krøyer

Eurystheus tenuicornis Holmes

Family Ampithoidae

Ampithoe simulans sp. nov.

Ampithoe valida (S. I. Smith)

Family Podoceridae

Podocerus spongicolus sp. nov.

SYSTEMATIC DISCUSSIONS

Where new species are described, a short diagnosis is given before the more detailed description. Under the heading of *Remarks* will be found any facts of general biologic interest noted at the time of collection, and a brief discussion of the approximate position of the species in the genus.

The holotypes will be found in the collection of the United States National Museum at Washington, D. C. Paratypes are deposited in the collection of the Department of Zoölogy of the University of California.

Inasmuch as comparatively little systematic work has been done on the Amphipoda of the Pacific Coast, it has been thought advisable to establish new species wherever constant structural differences were found to occur between our forms and the most closely related known forms. This will undoubtedly facilitate future systematic work when more complete collections are available.

Family Phoxocephalidae

Genus *Pontharpinia* Stebbing*Pontharpinia obtusidens* sp. nov.

(figs. 1-13 and 19)

Diagnosis.—Cutting edge of mandible (fig. 19) blunt; spine row with ten short, curved spines. Second joint of palp straight, equal in length to third. First joint of maxillary palp half as long as second. Plates of second maxillae narrow, with nine curved setae along the mediobasal border of the inner. Second joint of fifth pereopod (fig. 9) not overlapping third joint to any great extent.

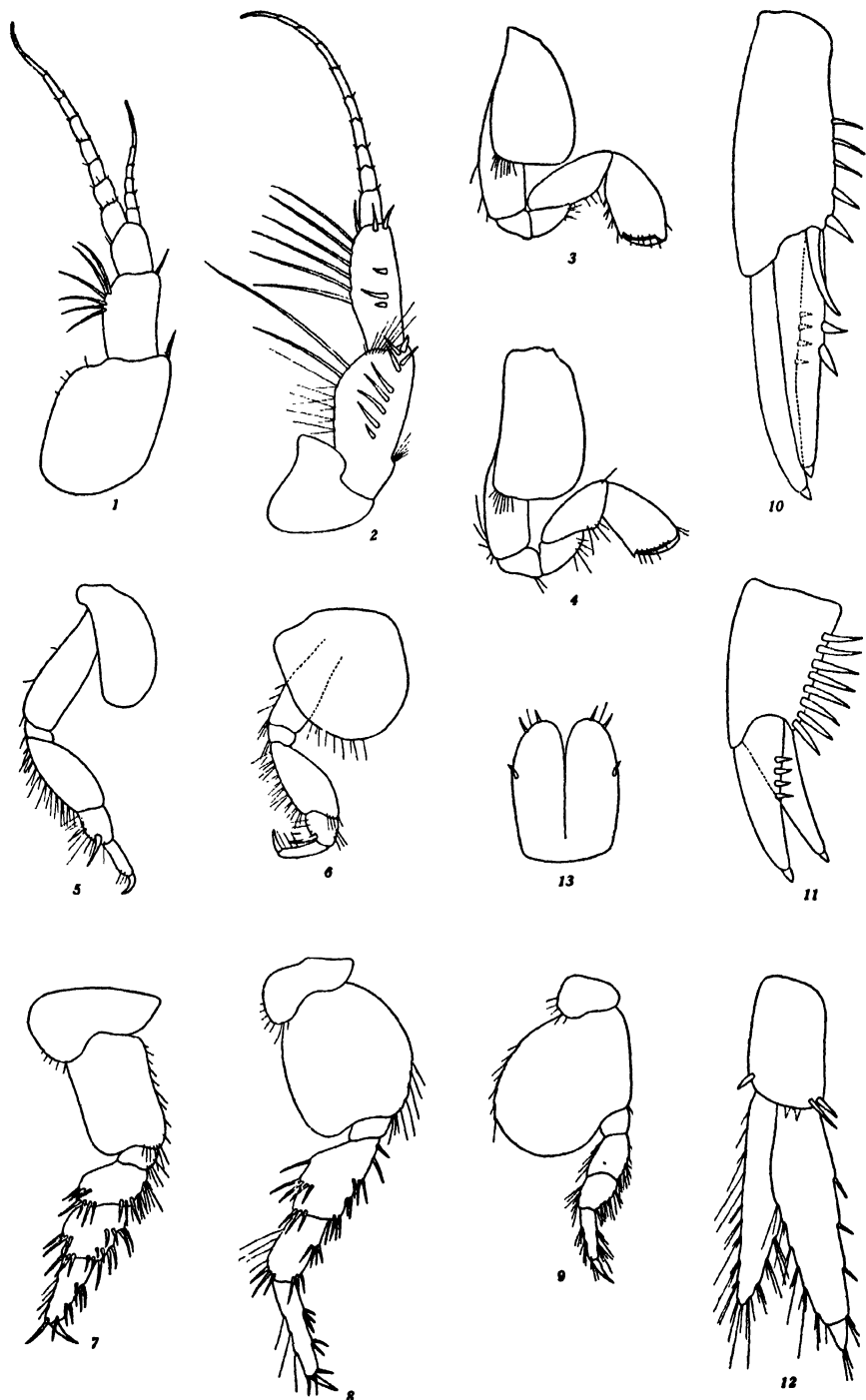
Description.—Rostrum hoodlike, reaching as far as second joint of peduncle of first antenna. Body smooth. Posterolateral corners of pleon segment 3 squarely rounded, with setae above angle. First side plate (fig. 3) slightly expanded distally; fourth the deepest and widest (fig. 6). Fifth bilobed; hind lobe the deeper (fig. 7). All with setae at posteroventral corner. Eyes black, subovate, flattened in front. First antennae in males similar to those of females (fig. 1); first joint very stout, twice as wide as second, which bears a group of slender spines on its ventrolateral margin. Third joint half as long as second. Flagellum 11-jointed, not twice as long as the 9-jointed accessory flagellum. Second antenna in female (fig. 2) turned back laterally, spinose; the penultimate and ultimate joints of the peduncle bearing lateral rows and apical groups of spines and setae. Flagellum twice as long as last joint of peduncle in female; in male almost six times as long, and bearing calceoli on alternate joints.

First and second gnathopods (figs. 3 and 4) similar in both males and females. Second joint twice as long as sixth. Sixth oblong, somewhat longer than fifth. Palm oblique, defined by a small acute tooth, minutely setose. First and second pereopods (figs. 5 and 6) similar; second joints not expanded; third joints very short; fourth joints widest, setose on hind margins. Fifth joint one-half as long as fourth, with a heavy spine on its lower hind corner.

Pontharpinia obtusidens ♀

- Fig. 1. First antenna, × 50
 Fig. 2. Second antenna, × 50
 Fig. 3. First gnathopod, × 33
 Fig. 4. Second gnathopod, × 33
 Fig. 5. First pereopod, × 20
 Fig. 6. Second pereopod, × 20
 Fig. 7. Third pereopod, × 20

- Fig. 8. Fourth pereopod, × 20
 Fig. 9. Fifth pereopod, × 20
 Fig. 10. First uropod, × 55
 Fig. 11. Second uropod, × 55
 Fig. 12. Third uropod, × 55
 Fig. 13. Telson, × 50



(For explanation of above figures see bottom of page 54)

Second joint of third periopod (fig. 7) oblong, the sides parallel. Fourth and fifth joints expanded, bearing setae on fore and hind margins, as does the sixth. Periopod 4 (fig. 8) the longest; second joint much expanded, oval; fourth joint expanded; fifth not so much so. Last three joints heavily armed with spines and setae. Second joint of fifth periopod (fig. 9) much expanded posteriorly, but not overlapping third joint; the rest of the appendage reduced and comparatively weak.

Cutting edge of mandible (fig. 19) blunt; molar absent. Spine row with ten short curved spines. First joint of palp one-fourth as long as second. Second straight, equal in length to third. Third with setae at the obliquely truncate apex. Inner plate of maxillipeds reaching as far as apex of first joint of palp, with a single conical spine tooth and many setae at apex, and with a row of long medial setae. Outer plate reaching halfway to apex of second joint of palp, armed with ten microscopically plumose spines along mediodistal margin. Palp strong; second joint almost twice as long as third. Fourth clawlike, almost as long as third. Inner plate of first maxilla rounded, with three or four apical setae. Outer plate with nine spines, the outer of which is the longest. Palp somewhat obscurely 2-jointed; the first joint half as long as the second, which bears a few apical setae. Plates of second maxillae narrow; the outer the longest and slightly the widest. Inner plate with nine curved setae along distomedial margin. Lower lip with inner lobes fused to outer. Upper lip rounded below. Epistomal projection low, ridgelike.

Outer ramus of first uropod (fig. 10) somewhat longer than the inner; rami approximately equal in length to peduncle, and bearing strong dorsal spines. Peduncle with a dorsal row of strong curved spines. Rami of second uropod (fig. 11) equal to each other and to the peduncle; the outer bearing a dorsal row of four conical spines. Peduncle with a similar row of eight spines. Rami of third uropod (fig. 12) lanceolate and notched laterally. Outer ramus the longer, with spines along lateral margin and setae along medial margin, and with a minute terminal joint. Inner ramus with setae along both margins. Peduncle with a row of short spines at its ventrolateral apex. Telson (fig. 13) cleft almost to base; the apices rounded, and bearing a single lateral and two subterminal spines, the latter with a single seta between them.

Length of females 9 mm.; of males 5 mm. Color whitish, opaque.

Remarks.—Two females and three young (?) males taken from kelp hold-fasts.

This species is very closely related to *P. rostrata* (Dana), differing principally in the characters of the mouth parts and in the second joint of the fifth periopod, which in *P. rostrata* overlaps the third and fourth joints.

No previous record of this genus has been made on the Pacific Coast.

Family Pleustidae

Genus *Pleustes* Bate

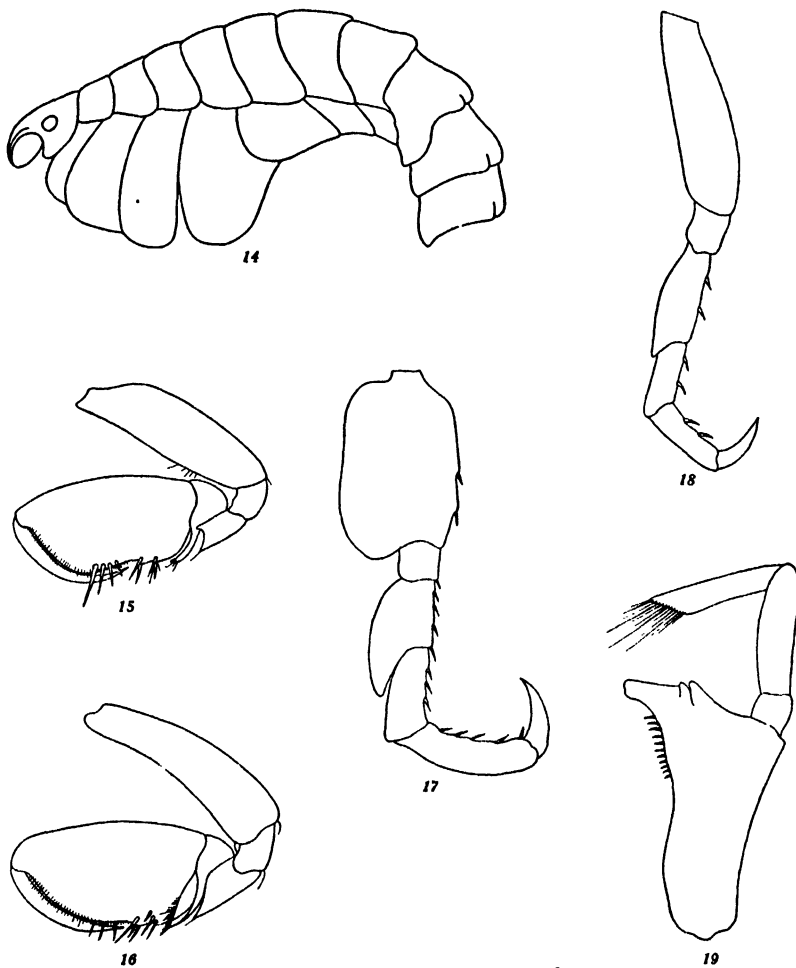
Pleustes depressus sp. nov.

(figs. 14–18)

Diagnosis.—Body (fig. 14) tricarinate, but carinae low and almost obsolete. Rostrum large, extending beyond end of peduncle of first antennae, rounded above, keeled below. Flagellum of first antenna three times as long as peduncle, 15-jointed. Flagellum of second antenna equal in length to peduncle, 13-jointed. Telson flat, not constricted at base, rounded apically.

Description.—Body (fig. 14) with a mediodorsal and two dorsolateral carinae, beginning on the sixth perion segment. Carinae poorly defined. Dorsal carina extending as far back as the second segment of the abdomen; the lateral carinae to the last segment of the abdomen. Rostrum as above. Lateral lobes of head not produced. Eyes round, black in alcohol. Side

plates 1-4 very deep; fourth acute-angled below the deep emargination. First antennae little more than half again as long as the head, little longer than second antennae. First joint of peduncle of first antenna as long as second and third combined. Flagellum three times as long as peduncle, 15-jointed. Antepenultimate joint of peduncle of second antenna the shortest, two-thirds as long as the penultimate. Ultimate and penultimate joints equal. Flagellum equal in length to peduncle, 13-jointed.



Figs. 14-18. *Pleustes depressus* ♀

Fig. 14. Side view of head, perion, and first three segments of pleon, $\times 11$
 Fig. 15. First gnathopod, $\times 33$

Fig. 16. Second gnathopod, $\times 33$
 Fig. 17. Fourth periopod, $\times 33$
 Fig. 18. Second periopod, $\times 33$

Fig. 19. *Pontharpinia obtusidens* ♀

Fig. 19. Mandible, $\times 55$

First gnathopod (fig. 15) slightly smaller than second. Sixth joint twice as long as wide. Palm oblique, defined by a series of spines. Finger very long and slender, curved, overlapping palm. Fifth joint short, its hind margin produced into a fingerlike process separating fourth and sixth joints. Fourth joint with acute lower hind margin. Third joint with a distal lobe on inner margin. Second gnathopod (fig. 16) similar to first, except that the finger-

like process of the fifth joint is flattened more or less cup-fashion over the base of the sixth joint. First and second periopods (fig. 18) similar. Second joint slender; finger pointing backwards; the fourth, fifth and sixth joints armed posteriorly with a few short spines. Third, fourth, and fifth periopods similar (fig. 17). Second joint expanded, three times as wide as the third, oblong, half again as long as wide. Fourth joint somewhat expanded, produced into a lobe at the lower hind corner. All joints armed anteriorly with spines. Finger pointing forward.

Maxillipeds with outer plate not reaching apex of first joint of palp; inner plates half as long. Fourth joint of palp clawlike. Inner plate of first maxilla low, with a single subapical seta. Outer plate little higher than wide, with nine spines. Palp 2-jointed; first joint triangular, one-third as long as the second, with a single spine at its somewhat produced outer apex. Second joint armed with apical and subapical spine teeth, and with microscopically plumose hairs along the outer edge. Inner plate of second maxilla wider than outer. Both sparsely armed with setae. Mandible with dentate cutting edge. Spine row with many short spines. Molar obsolete. Palp with first joint half as long as second; second and third about equal. Third joint slightly curved, with setae along inner margin of curve. Lower lip with poorly defined inner lobes. Upper lip bilobed. Epistomal process well marked, triangular, knifelike in front.

Rami of first uropod equal in length to each other and to the peduncle. Outer ramus of second uropod two-thirds as long as inner, equal in length to peduncle. Outer ramus of uropod 3 half as long as inner, half again as long as peduncle. All rami lanceolate, and with the peduncles, spinulose. Telson flat, not constricted at base, rounded apically, five-sevenths as wide as long.

Length of one specimen 6 mm.; of the other 8 mm. Color light tan.

Remarks.—Two females, taken from kelp holdfasts in two fathoms of water.

The specimens in my collection differ from *P. panoplus* (Krøyer) in that the rostrum is not hollowed dorsally, and in the flattened telson. They differ from *P. cataphractus* (Stimpson) in that the second joint of periopods 3, 4, and 5 is well expanded. The marked weakness of the carinae differentiates them from *P. panoplus*, *P. cataphractus*, and *P. occidentalis* (Stimpson).

The only previous record of this genus from the Pacific Coast is that of Stimpson (1864), from Puget Sound. His *Ampithonotus occidentalis* has been referred to the genus *Pleustes* by Stebbing (1906).

Family Atylidae

Genus *Nototropis* A. Costa

Nototropis tridens sp. nov.

(figs. 20-25)

Diagnosis.—Fourth, fifth, and sixth segments of pleon (the latter two fused) each bearing a dorsal tooth (fig. 22). Rostrum acute, curved down, half as long as the first joint or antenna 1. Second joint of third periopod (fig. 23) with a very slight, rounded lobe at its lower hind corner, not reaching beyond articulation of second and third joints. Second joint of periopod 4 (fig. 24) without a process. Second joint of fifth periopod (fig. 25) produced into a lobe at its lower hind corner, reaching to the articulation of third and fourth joints. Rami of third uropod three times as long as peduncle. Color transparent, whitish, without markings of any sort.

Description.—Rostrum and pleon segments as above. Posterolateral corners of first, second, and third pleon segments acute, with a lateral oblique ridge ending at the acute point. Pleon segment 4 acute below the articulation of the first uropod. Eyes black, reniform, very large, being equal in height to the length of the first joint of the first antenna. Side plates

generally higher in female than in male. First three side plates subquadrate, rounded below, the first widened somewhat distally. Fourth side plate slightly excavate behind. Fifth and sixth each with two emarginations on lower edge (fig. 22). Seventh shallow, excavate slightly below. First and second joints of peduncle of first antenna subequal; first the stouter. Third joint one-third as long as second. Flagellum half again as long as peduncle, 16-jointed. Second antenna longer and more slender than first. Penultimate joint of peduncle more than twice as long as antepenultimate; ultimate more than three times as long. Flagellum equal in length to peduncle, 19-jointed.

Second joint of first gnathopod (fig. 20) little longer than sixth. Sixth joint half again as long as the triangular fifth, and slightly wider; with several series of spines. Finger half as long as sixth joint, stout, matching palm. Second joint of second gnathopod (fig. 21) two and one-half times as long as sixth. Second gnathopod otherwise similar to first, but more attenuate. First and second periopods similar. Second joint not expanded. Sixth joint two and one-half times as long as fifth, slightly longer than second. Claw strong, directed backward. Second joint of third, fourth, and fifth periopods expanded; mostly so in the fifth. Second joint of third periopod (fig. 23) with unarmed front margin and with two or three spines on hind margin. Posterodistal lobe obscure. Other joints somewhat shorter, but similar to those in the first and second periopods. Second joint of fourth periopod (fig. 24) half again as wide and long as that of the third, armed with a few spines on front margin and many behind; posterodistal process completely absent. Second joint of fifth periopod (fig. 25) half again as high as third, armed with spines both anteriorly and posteriorly. The lobe-like process at the posterodistal corner reaches the articulation of the third and fourth joints.

Inner plates of maxillipeds half as long as outer. Outer plates reach past apex of second joint of palp. Fourth joint of palp clawlike, its insertion into the third subapical. Inner plate of first maxilla well developed, obliquely truncate at apex, which bears four plumose spines. Outer plate more than twice as long as inner; apex with eleven spines. Palp 2-jointed. Inner plates of second maxillae narrower than outer, with spines along distal margin and distal half of medial margin. Mandibles with prominent molar; spine row with five or six plumose spines. First joint of palp short, one-fourth as long as second, one-third as long as third. Inner lobes of lower lip fused to outer. Mandibular processes slender, slightly twisted at apex. Upper lip rounded below.

Rami of first and second uropods tipped with a spine, and with spines along their medio-dorsal margins; slender, not lanceolate. Rami of first uropod equal, three-fourths as long as peduncle. Inner ramus of second uropod longer than outer, subequal to peduncle. Rami of third uropod subequal, flattened, and lanceolate, armed with setae toward tip, three times as long as peduncle. Telson longer than wide, cleft nearly to base; each half with a small notch at apex, which bears a minute spine.

Length 10–11 mm. Color whitish, translucent.

Remarks.—Many males and females, taken by means of an electric light trap at night. It was also possible to take them by means of a surface tow between nine and eleven o'clock at night. Surface tows at other times were unsuccessful, indicating a peak about ten o'clock.

This form is quite close to *N. swammerdamei* (Milne-Edwards) but differs in the characters of the second joints of the last three periopods, which in *N. swammerdamei* all bear posteroventral processes. It also differs in the dentition of the pleon, which bears three dorsal teeth rather than one.

This appears to be the first record of any member of the family Atylidae on the Pacific Coast. The closely related species *Nototropis swammerdamei* appears, however, to have a very wide distribution, having been reported from the Mediterranean, the Arctic Ocean and the North Atlantic.

Family Gammaridae
Genus Maera Leach
***Maera inaequipes* (A. Costa)**

Remarks.—This was one of the most commonly encountered amphipods on the kelp holdfasts. The specimens agree in general with the description given by Stebbing (1906) except that the gland cone on the second antenna does not reach beyond the antepenultimate joint of the peduncle, periopods 1–5 bear a moncuspidate rather than a tricuspidate finger, and the animals are larger: 9–12 mm. rather than 7 mm. It is the opinion of the writer that these minor differences are not sufficiently great to justify the erection of a new species.

Chilton (1917), in discussing the distribution of this species, notes that it has been reported by various observers from the Mediterranean, the North Atlantic, Ceylon, Australia, New Zealand, Cape Horn, and Chile. The distribution almost coincides with that of *Elasmopus rapax* (A. Costa). *Elasmopus rapax* was not found at Moss Beach, but *E. brasiliensis* (Dana) occurred in large numbers in the same ecologic niche as *Maera inaequipes*.

This is the first record of this species from the Pacific Coast. Previous records of the genus have been made by Bate (1864) from Vancouver Island, Calman (1898) from Puget Sound, Holmes (1904) from Puget Sound, Holmes (1908) from Monterey Bay and Santa Barbara Island, Stimpson (1856 and 1857) from San Francisco Bay, Stout (1913) from Laguna Beach.

Genus Elasmopus A. Costa
***Elasmopus brasiliensis* (Dana)**

The specimens taken correspond with the description given by Stebbing (1906) except that the flagellum of the first antenna is somewhat shorter than the peduncle, and the accessory flagellum is 2-jointed instead of 3-jointed.

Both males and females were common on the kelp holdfasts.

The only previous record of the genus *Elasmopus* from this coast is that of Shoemaker (1925). Since only a single female specimen was taken in the Gulf of California, the species was not determined.

Genus Melita Leach
***Melita californica* sp. nov.**

(figs. 26–32)

Diagnosis.—Pleon segment 4 (fig. 26) with a median dorsal and two subdorsal teeth. Pleon segment 5 with four subdorsal teeth. All other segments without teeth. Finger of

Figs. 20–25. *Nototropis tridens* ♂

Fig. 20. First gnathopod, × 33

Fig. 21. Second gnathopod, × 33

Fig. 22. Side view of head, perion, and pleon, × 9

Fig. 23. Second joint of third periopod, × 33

Fig. 24. Second joint of fourth periopod, × 33

Fig. 25. Second joint of fifth periopod, × 33

Figs. 26–30. *Melita californica*

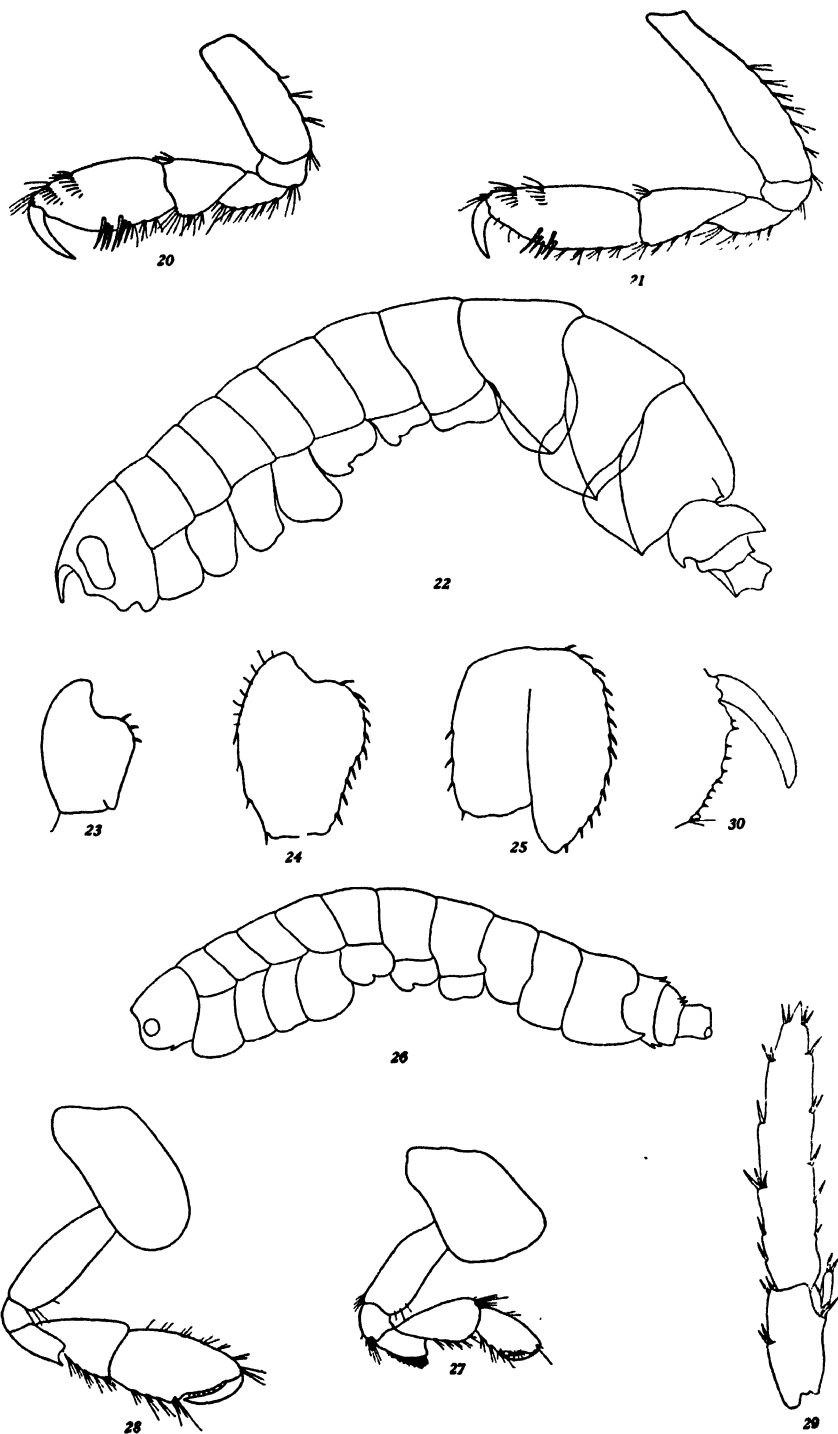
Fig. 26. Side view of head, perion, and pleon, × 9

Fig. 27. First gnathopod ♀, × 33

Fig. 28. Second gnathopod ♀, × 33

Fig. 29. Third uropod, × 33

Fig. 30. Palm of gnathopod 2, ♂, × 33



(For explanation of above figures see bottom of page 60)

second gnathopod in male (fig. 31) subacute, not closing on palm, but against inner surface of sixth joint. Outer ramus of third uropod two and one-half times as long as peduncle. Inner ramus very small, lamellar.

Description.—Rostral point small. Lateral corners of head broadly rounded. Eyes round. Postantennal corners with a small tooth. Side plates 1–4 about equal in depth, rounded below; fourth the widest, excavate behind. First side plate flaring distally; fifth and sixth bilobed; seventh obscurely so. Fourth pleon segment with a median dorsal and two subdorsal teeth. Pleon segment 5 with four subdorsal teeth. First antenna somewhat more than half as long as body. Second joint of peduncle somewhat longer than first; third half as long. Flagellum 20-jointed; as long as peduncle. Accessory flagellum one-fourth as long as primary, 8-jointed. Second antenna four-fifths as long as first. Penultimate joint of peduncle a little longer than ultimate, three times as long as antepenultimate. Gland cone reaching two-thirds the length of the antepenultimate joint. Flagellum 12-jointed.

Fifth joint of first gnathopod in male (fig. 32) longer than sixth. Sixth more than twice as long as wide. Palm oblique. Fourth joint with a thick brush of closely set setae on its hind margin. Fifth joint with the setae microscopically plumose. First gnathopod in female (fig. 27) like that in male except that the sixth joint is not twice as long as wide and the whole appendage is comparatively smaller. Second gnathopod in the male (fig. 31) with fourth joint acutely produced at the lower hind margin into a hooklike tooth. Fifth joint subtriangular; sixth joint not twice as long as wide, ovate. Finger subacute, not closing on palm, but against inner surface of sixth joint. Palm (fig. 30) crenulate, with short spines. Second gnathopod in female (fig. 28) similar to that in male except that it is smaller and that the fourth and fifth joints are proportionally longer. Finger closing on the palm. First and second periopods similar, slender. Second joint narrow. Third joint very short. Third periopod not reflexed, more slender than fourth or fifth. Second joint expanded to a suboval plate in periopods 3, 4, and 5, the fifth being the widest. Fourth joint quite heavy in the fourth and fifth periopods, which are equal in length.

Maxillipeds normal. Fourth joint of palp acute; equal in length to third. Outer plates almost reaching apex of second joint of palp. Inner plates half as long as outer. Inner plates of first maxillae well developed, wider than outer; apices acute, armed with twelve spines along inner margins. Outer plate truncate at apex, armed with nine multidentate spines. First joint of palp more than one-third as long as second. Outer and inner plates of second maxillae about equal. Inner fringed along inner margin with a double row of setae. Mandibles with quadridentate cutting edge; molar and spine row normal. Palp slight, the third joint three times as long as the first, slightly longer and more slender than the second. Inner lobes of lower lip prominent. Upper lip rounded below.

First uropod longer than second; the rami equal, and equal to the peduncle. Outer ramus of second uropod slightly shorter than inner, equal to the peduncle. Peduncle of uropod 3 (fig. 29) about equal to that of the second, but with the outer ramus much elongated; two and one-half times as long as the peduncle, furnished with a single stout conical spine at the tip, and with several groups of lateral spines. Inner ramus very small, lamellar, tipped with two spines. Telson flat, two-thirds as long as peduncle of third uropod, cleft to the base; each half with two subapical spines, an apicolateral and a small medial spine.

Length 9–11 mm. Color grayish black.

Remarks.—Large numbers of males and females, many of them clasping, taken under stones in the intertidal zone.

This species differs from *Melita obtusata* (Montagu) in that the finger of gnathopod 2 in the male is subacute and does not close on the palm. Moreover, the lower hind corners of side plates 1–3 are without a denticle as in *M. obtusata*. It differs from *M. amoena* Hansen in that the outer ramus of uropod 3 is much more than twice as long as the peduncle, and that the color is grayish black rather than whitish. Yet it is closely related to both these species.

Two previous records of this genus have appeared from the Pacific Coast. Stimpson (1856) described *Maera confervicola* from the San Francisco Bay region, and later (1857) placed it in the genus *Gammaropsis*. It has been referred to the genus *Melita* by Stebbing (1906). The second report is that of Walker (1898) who records *Melita dentata* (Krøyer) from Puget Sound.

Family Dexaminidae

Genus *Polycheria* Haswell

Polycheria antarctica (Stebbing)

Remarks.—The Moss Beach *Polycheria* agrees very closely with Calman's (1898) original description of *P. osborni* from Puget Sound, and that of Skogsberg and Vansell (1928) from Monterey Bay, California. We have accepted the decision of Chilton (1912), which was based on careful museum studies of all known species of *Polycheria*, including the Pacific Coast species. Since he concluded that the differences between them are negligible, we must consider the Pacific Coast species to be *P. antarctica*.

Our specimens were found, as were those of Calman, and of Skogsberg and Vansell, in cavities in the compound ascidian *Amaroucium* sp. The habits noted agree in detail with those described by Skogsberg and Vansell. Additional facts noted were that the hooklike process of the third side plate seems to be used, along with the third periopods, in holding the animal in its burrow; also that the species may often occur in the siphons of *Clavellina* sp., although Skogsberg and Vansell believed, as a result of laboratory trials, that *Amaroucium* was the only acceptable host.

One female with eggs was taken at Moss Beach from a kelp holdfast. They were exceedingly numerous in a small sea-cave near Montara, San Mateo County, California, the walls of which were covered with tunicates and sponges of many types.

Family Aoridae

Genus *Aoroides* A. Walker

Aoroides californica sp. nov.

(figs. 33–38)

Diagnosis.—First antenna without accessory flagellum. Lateral lobes of head (fig. 36) prominent. Flagellum of second antenna with 3-jointed flagellum. Cutting edge of mandible pentadentate. Last joint of palp with two apical setae; penultimate joint without setae. Ultimate joint half again as long as penultimate. Outer ramus of third uropod longer than inner; inner with two spines on inner margin.

Description.—Rostral point absent. Anterolateral lobes pronounced, rounded. Eyes black in alcohol, oval to slightly kidney-shaped. Posterolateral corners of pleon segment 3 subquadrate. First side plate in male (fig. 33) greatly extended, two and one-half times as long as the depth of side plate 2. Second side plate in male (fig. 34) similar to third and fourth, not produced forward. First side plate in female (fig. 35) higher than wide, also slightly extended forward. Side plates 3 and 4 similar, rounded below. Fifth bilobed, the hind lobe more than half as deep as the front lobe. Sixth similar to fifth, but smaller. Seventh rounded, somewhat excavate in front. First joint of first antenna heavy, four-fifths as long as the

very slender second, and almost three times as long as the third. Flagellum slender, longer than peduncle, 23-jointed. Accessory flagellum absent. Second antenna much stouter than first, three-fifths as long. Last two joints of peduncle subequal, almost three times as long as third joint of peduncle, 3-jointed, the last two joints very short.

First gnathopod in male (fig. 33) greatly elongate, about three-fourths as long as body, complexly subchelate. Second joint long and slender, narrower at base than at apex. Fourth joint produced into a long spinous process, sharp-pointed and longer than sixth joint. Finger with many setae on inner surface. Palm undefined. First gnathopod in female (fig. 35) about as long, but much heavier than second. Fifth joint attached to anterior side of fourth. Palm undefined, the combined palm and hind margin bearing a thick spine about midway between the articulation of the fifth and sixth joints and the finger hinge. Finger serrate. Second gnathopod in male (fig. 34) with elongate second joint, densely setose along its anterior margin. Palm oblique, defined by a spine. Finger smooth. Second gnathopod in female (fig. 38) with second joint elongate, a few setae along its anterior margin. Palm oblique, defined by a spine. Finger serrate. First and second periopods similar; second joint slender. Third and fourth periopods similar. Second joint expanded and serrate posteriorly for the reception of setae. Fifth joint equipped with four apical and three lateral spines in the third periopod (fig. 37), with four apical and two lateral spines in the fourth periopod. Fifth periopod the longest; second joint expanded, but fifth joint without spines.

Fourth joint of palp of maxillipeds clawlike. First maxilla with 2-jointed palp, which is broader than the outer plate. Inner plate obsolete, bearing a single seta. Inner plate of second maxilla with both apical and lateral setae. Cutting edge of mandible pentadentate. Accessory plate present on both sides; spine row with two plumose spines. Molar normal. Palp very weak, not as long as mandible from base to cutting edge. Second joint twice as long as first, without setae. Third joint three times as long as first, with two apical setae. Lower lip normal; inner lobes well developed. Upper lip rounded below.

Rami of first uropod equal in length to each other and to peduncle, which bears a long spine extending between the two. Both rami with dorsal and apical spines. Inner ramus of second uropod half again as long as peduncle; longer than outer ramus. Peduncle with apical spine similar to that in uropod 1. Outer ramus of third uropod slightly the longer, with terminal spines only, equal to peduncle, which bears two short apical spine teeth. Inner ramus with two spines along inner margin. Telson truncate at apex, the lateral edges of the apex being raised into little points; with two lateral setae on each side.

Length 6-8 mm. Color salmon-pink, translucent, with small black pigment spots evenly scattered over body.

Remarks.—This genus was described by A. O. Walker on the basis of nine females taken in Puget Sound. The male of the type species *A. columbiae* has remained unknown, and no further reports of the genus have been made.

The specimens taken at Moss Beach differ in certain details from the description given by Walker (1898). For example, the lateral lobes of the head are quite prominently produced in ours, whereas in *A. columbiae* they are not well marked. The flagellum of the second antenna is 3-jointed instead of 8-

Figs. 31-32. *Melita californica*

Fig. 31. Second gnathopod, medial view, ♂, × 33

Fig. 32. First gnathopod, lateral view, ♂, × 33

Figs. 33-38. *Aoroides californica*

Fig. 33. First gnathopod, ♂, × 12

Fig. 36. Head, lateral view, × 33

Fig. 34. Second gnathopod, ♂, × 33

Fig. 37. Fifth joint of third periopod, × 85

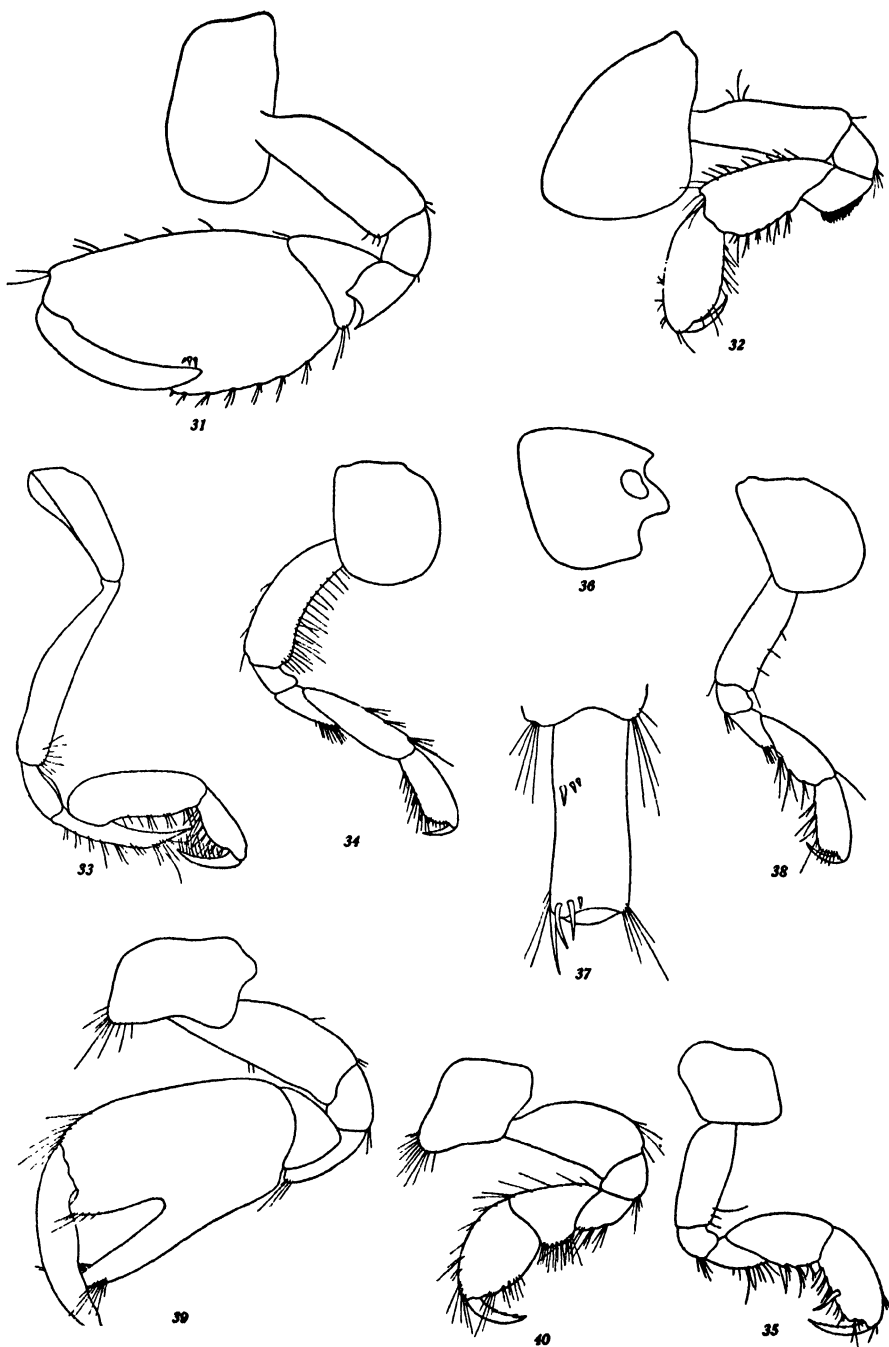
Fig. 35. First gnathopod, ♀, × 33

Fig. 38. Second gnathopod, ♀, × 33

Figs. 39-40. *Photis conchicola* ♂

Fig. 39. Second gnathopod, × 33

Fig. 40. First gnathopod, × 33



(For explanation of above figures see bottom of page 64)

jointed. These and other minor differences have led the writer to erect a new species. Collections from Oregon and the coast of Washington may in the future show ours to be the same species as that found in Puget Sound.

Family Photidae

Genus *Photis* Krøyer

Photis conchicola sp. nov.

(figs. 39-43)

Diagnosis.—First joint of peduncle of first antennae equal to second, half again as long as third. Palm of first gnathopod oblique, poorly defined. Second gnathopod in male (fig. 39) very large; sixth joint much longer than second, the hind margin continuous into an elongate tooth which is half as long as the sixth joint. Finger overlapping palm by one-third its length. First and second side plates in the male (figs. 39-40) wider than deep. Fourth side plate (fig. 42) in both male and female wider than the front lobe of the fifth (fig. 43). Second joint of third periopod (fig. 43) heart-shaped, longer than broad. Peduncle of third uropod more than twice as long as outer ramus. Inner ramus one-third as long as outer. Telson triangular.

Description.—Body stout; heavier in female than in male. Rostrum a minute point. Eyes round, on subacute lateral lobes. Second antenna but slightly longer than first. First and second side plates in male (figs. 39-40) much shallower than the rest, produced forward, and bearing setae at their anteroventral corners. First and second side plates in female not marked off from the rest by their shallowness, the first five side plates forming a graded series with the third the deepest and widest. Fourth three-fourths as wide as third, its average width greater than that of the anterior lobe of the fifth. Posterior lobe of fifth more than one-third as deep as anterior lobe. Sixth and seventh side plates shallower than hind lobe of fifth; the hind margin of the seventh scalloped. First joint of first antenna stout, equal in length to second; third joint two-thirds as long as second. Flagellum 7-jointed, the joints about equal in length, and the whole flagellum equal in length to the second and third joints of the peduncle. The ultimate joint bears two curved spines. Ultimate and penultimate joints of peduncle equal; ultimate twice as long as antepenultimate. Flagellum 7-jointed, somewhat shorter than last two joints of peduncle combined.

First gnathopod (fig. 40) similar in male and female; second joint longest; sixth widest. Palm oblique, poorly defined, slightly crenulate. Finger serrate. Second gnathopod in male (fig. 39) abnormally large; sixth joint much longer than second, the hind margin being continuous into an elongate tooth which is half as long as the sixth joint. Palm very deeply excavate behind this tooth, rising to a short rounded tooth nearer the finger. Finger almost straight, overlapping the palm. Fifth joint almost completely fused to sixth, cup-shaped. Sixth joint of second gnathopod in female one and three-fifths times as long as broad. Palm oblique, excavate, defined by a palmar spine. Fifth joint produced into a lobe at posterodistal corner; second with a lobe at anterodistal corner. Second joint of first and second periopods not expanded. Third periopod (fig. 43) reflexed, the second joint heart-shaped, longer than broad; claw reduced. Fourth and fifth periopods similar, about equal in length. Second joints expanded and scalloped along hind margin.

Maxillipeds normal. Fourth joint of palp short, rounded, armed with setae. Outer plate armed along its distal and inner margins with sixteen spines as well as setae. Inner plates truncate at apex, armed with plumose setae. Inner plate of first maxilla well developed, half as long as outer plate, but unarmed. Outer plate truncate at apex, armed with eleven spine-teeth. Palp 2-jointed; first joint one-fifth as long as second. Outer plate of second maxilla oval, longer and wider than inner, which is armed both distally and medially with setae. Lower lip with prominent inner lobes. Mandible with cutting edge and accessory plate bidentate on left. Spine row with four plumose spines and many short hairs. Accessory plate ab-

sent on right, which has a quadridentate cutting edge and four spines in the spine row. Molar well developed. Upper lip slightly emarginate below. Epistomal process triangular, fused to lower side of head.

Peduncle of first uropod equal in length to fourth and fifth segments of abdomen. Outer and inner rami somewhat more than half as long as peduncle. Peduncle of second uropod equal in length to fourth segment of abdomen. Outer ramus two-thirds as long as peduncle. Inner ramus almost equal in length to peduncle. Peduncle of third uropod equal in length to inner ramus of second. Inner ramus one-third as long as outer, tipped with a short conical tooth, a long serrate spine and a seta. Telson triangular, keeled ventrally.

Length 6 mm. First segment of antenna 1, head, first side plate, and anterior part of first dorsal sclerite dark reddish brown. Dorsal sclerites of fourth and fifth segments slightly darker than second and third. Rest of body dull yellowish.

Remarks.—Two males, six females taken from small snail shells which seem to be immatures of *Calliostoma* sp. The shells were obtained from kelp holdfasts recovered from two fathoms.

This species closely resembles *P. reinhardi* Krøyer, differing from it primarily in the great development of the tooth on the second gnathopod of the male, and in the reduction in height of the first two side plates of the male. Our specimens of *P. reinhardi* all show the fourth side plate narrower than the front lobe of the fifth, and by this method the females of *P. conchicola* and *P. reinhardi* may easily be separated, although other differences, except those of proportion of parts to one another, are difficult to find between the females of the two species. The males are, however, easily differentiated. *P. conchicola* differs from *P. californica* Stout in the shallowness of the first two side plates in the male.

Photis reinhardi Krøyer

Remarks.—This species was found very commonly on the kelp holdfasts, along with *P. conchicola*. Our forms differ only very slightly from the description given by Stebbing (1906) in lacking the small processes on each side of the apex of the telson.

This species has previously been reported from Monterey Bay, California, by Holmes (1908). Stout (1913) described a new species, *P. californica*, from Laguna Beach. No further records of the genus on this coast have been encountered.

Eurystheus tenuicornis Holmes

Remarks.—The original description of this species was made on the basis of immature specimens. Shoemaker (1930) in studying specimens from Monterey, California, was the first to observe the great development of the seventh side plate in the male, which sharply distinguishes it from all other species of the genus. The only other record of the genus from this coast is that of Holmes (1908), who described the new species *E. dentatus* from specimens taken in Alaska.

This species does not seem to be common at Moss Beach, only five specimens being taken there in three months. All these came from kelp holdfasts.

Family Ampithoidae

Genus *Ampithoe* Leach*Ampithoe valida* (S. I. Smith)

The only previous record of this species encountered is that of Smith (1873), who described it from specimens taken in Long Island Sound. Despite the great difference in locality, our specimens agree perfectly with the description given by Stebbing (1906). Inasmuch as this description concerns only the antennae and gnathopods, it has been thought best to redescribe the species.

Description.—Rostral point absent. Eyes small, subrotund. Lateral lobes of head rounded. First side plate somewhat produced forward. Third, fourth, and front lobe of fifth side plates about equal in depth. Hind lobe of fifth not one-third as deep as front lobe. Posterolateral corners of pleon segments 1–3 faintly produced into a small blunt tooth, transversely ridged. First and second joints of peduncle of first antennae subequal in males and females. Third joint less than one-third as long as second. Flagellum 50-jointed. Second antenna somewhat shorter than first. Penultimate joint of peduncle half again as long as ultimate, three times as long as antepenultimate. First joint of the 25-jointed flagellum equal in length to the next three.

First gnathopod somewhat more slender in female than in male. Fifth and sixth joints equal in length and width. Palm oblique; convex. Sixth joint of gnathopod 2 in male oblong, widening slightly distally. Palm transverse, with a broad low median tooth and with a rounded defining prominence. Finger much curved. Second joint with a lobe at its anterodistal corner. Fifth joint produced at hind margin. Fifth joint of second gnathopod in female produced as in male, shorter than sixth joint. Palm smooth, convex. Second joint as in male. Second joint in all periopods much widened, narrowing abruptly distally. Periopod 3 reflexed; claw turned out, short and much curved. Fourth and fifth periopods the longest, about equal in length.

Fourth joint of palp of maxilliped clawlike. Outer plate reaching well beyond apex of second joint of palp, finely pectinate along inner margin. Inner plate of second maxilla narrower and shorter than outer, completely armed with setae along its medial margin. Inner plate of first maxilla rudimentary. Outer plate heavy, armed apically with nine spines. Palp 2-jointed, the second joint four times as long as the first and rounded apically. Cutting edge of mandible multidentate. Accessory plate present on right side only. Spine row with six spines. First joint of palp very short; second and third joints equal. Third joint widening to the unilaterally convex apex, which is armed with a series of long spines. Inner lobes of lower lip long and slender. Outer lobes very deeply incised. Upper lip rounded below.

Outer ramus of first uropod somewhat shorter than inner, less than half as long as peduncle. Outer ramus of second uropod likewise shorter than inner, but somewhat more than half as long as peduncle. Inner and outer rami of third uropod equal; the outer tipped with two hooks. Telson slightly but abruptly narrowed basally, rounded apically.

Color bright lettuce-green; the whole body covered with extremely minute black pigment spots. Length 9–11 mm.

Remarks.—A single pair found in copulation on the base of a plant of *Halosaccion glandiformis*. The species is, however, not rare, as it has been taken in other collections from the same locality.

Ampithoe simulans sp. nov.

(figs. 44–47)

Diagnosis.—First and second antennae variable in relative length, but usually subequal. Flagellum of first antenna little longer than peduncle. Flagellum of second antenna 20-

jointed, equal to last two joints of peduncle. Eye small, round, black. Hind margin of wrist of gnathopod 1 in male (fig. 46) rounded; palm convex; hand wider than wrist. Hand of the female second gnathopod (fig. 45) twice as long as fifth joint; palm undefined. Hand of second gnathopod of male (fig. 47) not produced into a lobe beyond articulation of finger; with a large tooth defining the palm.

Description.—Rostrum a minute point. Eyes on slightly produced lobes, dark pigmented. First antenna one-third as long as body. First and second joints of peduncle equal in length; third not quite half as long as second. Flagellum slightly longer than peduncle, 21-jointed. Second antenna stouter than first. Ultimate joint of peduncle slightly shorter than penultimate, twice as long as antepenultimate. Flagellum equal to last two joints of peduncle, 20-jointed. First side plate extended forward so that front margin makes an angle of 45° with axis of body. Others subrectangular, rounded below; fifth the deepest.

Finger of gnathopod 1 in male (fig. 46) serrate, half as long as sixth joint. Palm convex, oblique, undefined, with a lobe at the finger hinge. Sixth joint twice as long as broad. Fifth joint not quite three-fourths as long as sixth. Second joint with a prominent transparent lobe at anterodistal corner. Gnathopod 1 in the female (fig. 44) with fifth joint not as long nor as wide as the sixth, otherwise as in male. Second gnathopod in male (fig. 47) with finger serrate. Palm very oblique, setose, defined by a large blunt process, without a lobe at the finger hinge. Sixth joint not quite twice as long as fifth. Second joint with a transparent lobe as in gnathopod 1. Second gnathopod of female (fig. 45) with palm undefined. In other respects similar to that of male.

First and second pereopods stout, similar; second joint twice as wide as third. Fourth joint produced downward at anterodistal corner. Third pereopod reflexed; second joint subcircular; claw turned outward. Pereopods 4 and 5 longer and more slender than the others; fifth the longest. Second joint in both three times as wide as the other segments, somewhat more than half as wide as long. Second joint of palp of maxillipeds with two rows of about twenty spines along inner margin. Third joint armed with spines on both inner and outer margins. Fourth joint rounded on end, tipped with a strong spine which is as long as the joint itself. Outer plate armed with fourteen spine teeth along inner margin, with six long curved spines along outer margin and a small tooth at apex, separating the two groups. Inner plates armed with a row of long spines along inner margin, continuing across apex; outer surface bare. Inner lobes of lower lip prominent. Upper lip rounded below. Inner plate of first maxilla rudimentary. Outer plate from base to tip of spines as long as the two segments of the palp, half as wide as long, armed at apex with eleven strong spines. First joint of palp one-fourth as long as second. Inner plate of maxilla 2 narrower and shorter than outer, armed medially and distally with slender setae. Mandible with dentate cutting edge, accessory plate on both sides, and spine row of six serrate spines. Palp strong. First joint shortest; second twice as long as first; third three times as long as first, armed along its mediobasal margin with several rows of long spines.

Uropods all extending an equal distance behind body. Rami equal. Peduncle of second twice as long as that of third. Rami of the first pair with single rows of short spines, tipped with a single short spine. Outer ramus of third uropod armed with two hooks. Inner ramus with two short dorsally subapical spines and an armament of apical setae. Peduncle with four closely set conical spines along dorsal apex. Telson armed with two longitudinally placed rows of setae, bluntly pointed, pentagonal in outline, the distal sides of the pentagon half as long as the lateral sides.

Length 12–14 mm. Antennae transparent greenish, mottled thickly with dark and light brown and some white. Upper halves of side plates and lateral edges of dorsum same as antennae. Lower halves of side plates translucent green. Gnathopods opaque greenish white.

Remarks.—Our individuals agree in general with the description of this species given by Stebbing (1906) of *A. cinerea* Haswell. The second antennae of ours are not conspicuously shorter than the first, however, and the eye, in-

stead of being almost colorless, is quite black. The general body color of *A. cinerea* is given as "ashy grey." *A. simulans* differs from *A. vaillantii* (H. Lucas) in that the flagellum of the first antenna is not twice as long as the peduncle, and the palm of gnathopod 1 is convex rather than straight or excavate. It differs from *A. rubricata* (Montagu) in that the tooth defining the palm in the male gnathopod 2 is much larger and more prominent than in that species. *A. intermedia* Walker differs from ours in that the flagellum of the second antenna is only 9-jointed and equal to the last segment of the peduncle rather than the last two segments. The second and third joints of the mandibular palp are equal; in ours the third is half again as long as the first. In the second gnathopod of the male of *A. intermedia* the sixth joint is prolonged into a lobe beyond the articulation of the finger, whereas in *A. simulans* it is not.

None of the species mentioned has been reported from the Pacific Coast, although the reports of other members of this genus are numerous.

Family Podoceridae

Genus *Podocerus* Leach

Podocerus spongicolus sp. nov.

(figs. 48-51)

Diagnosis.—Body not carinate. Rostrum acute, one-fifth as long as first joint of first antenna. Fourth joint of second gnathopod in male (fig. 49) markedly produced into a lobe at lower hind corner. Palm smooth. Fourth joint of gnathopod 2 in female (fig. 51) subacute at lower hind corner. Periopods 1-5 with spines on both margins of the sixth joint, arranged in equidistant fascicles along front and hind margins. Telson tipped with six spines.

Description.—Form flattened, but robust, not carinate. Rostrum acute, triangular, about one-fifth as long as basal joint of first antenna. Eyes prominent, forming large dorsolateral protuberances on the sides of the head, giving the animals the appearance of a dragon-fly nymph. Dorsal sclerites of first seven segments laterally produced beyond the articulation of the side plates. First side plate produced forward to an acute point, tipped with a spine. All others very small and almost hidden by the winglike extensions of the dorsal sclerites. First antenna half as long as second. First joint of peduncle heavy, three-fifths as long as the second, and almost as long as the third. Secondary flagellum 1-jointed. Primary flagellum of four joints, the first of which is equal to the other three. Antepenultimate joint of peduncle of second antenna stout and short, one-fourth as long as penultimate, one-fifth as long as ultimate. Flagellum 3-jointed, the joints armed with fine setae.

Sixth joint of first gnathopod in male (fig. 48) longer than fifth; in female (fig. 50) subequal to fifth. Palm much longer than hind margin. Finger serrate. Fourth joint of gnatho-

Figs. 41-43. *Photis conchicola* ♂

Fig. 41. Side plate 3 and second joint of first periopod, $\times 33$

Fig. 43. Side plate 5 and third periopod, $\times 33$

Fig. 42. Side plate 4 and second joint of second periopod, $\times 33$

Figs. 44-47. *Ampithoe simulans*

Fig. 44. First gnathopod, ♀, $\times 33$

Fig. 46. First gnathopod, ♂, $\times 33$

Fig. 45. Second gnathopod, ♀, $\times 33$

Fig. 47. Second gnathopod, ♂, $\times 33$

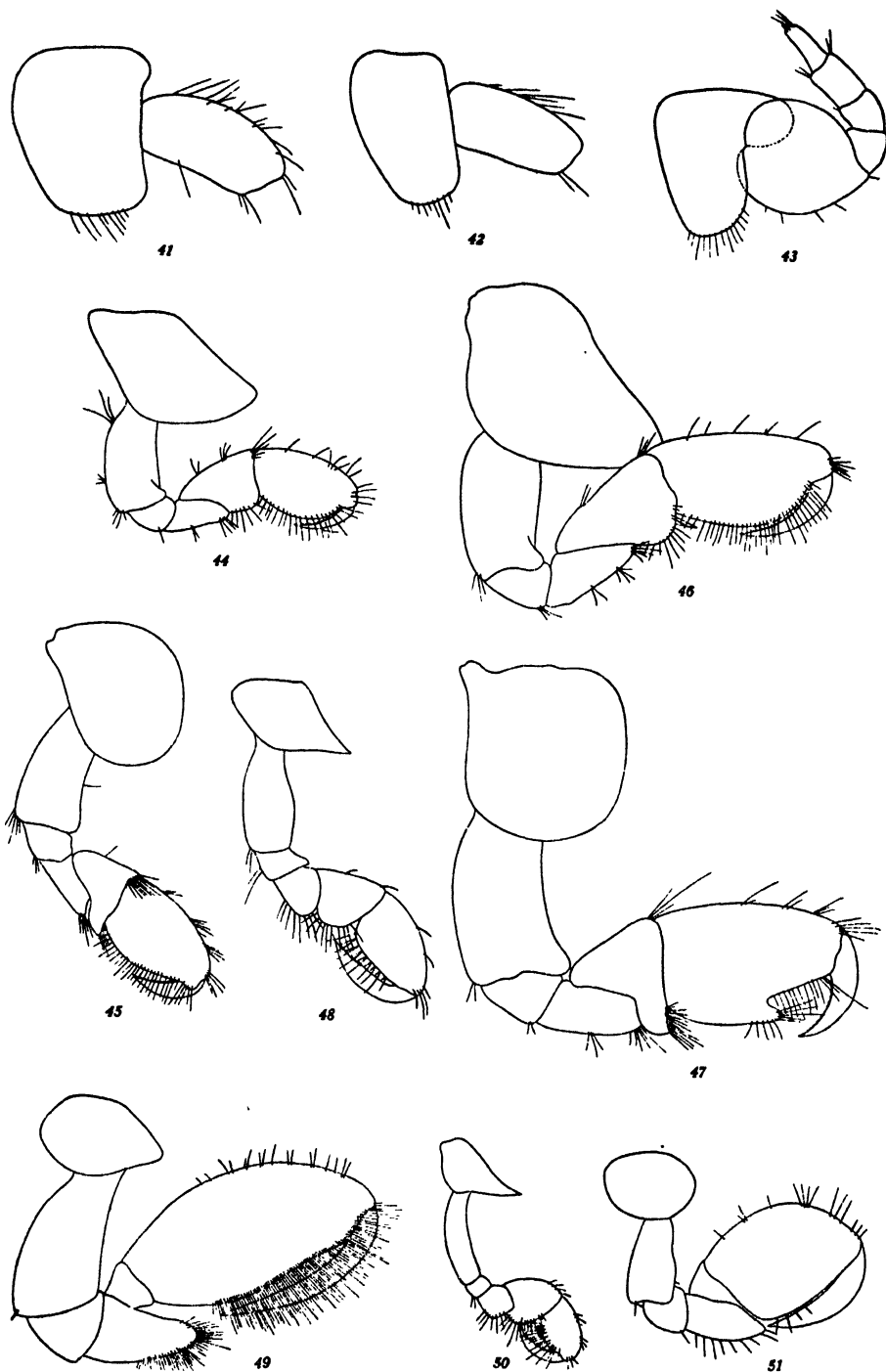
Figs. 48-51. *Podocerus spongicolus*

Fig. 48. First gnathopod, ♂, $\times 55$

Fig. 50. First gnathopod, ♀, $\times 55$

Fig. 49. Second gnathopod, ♂, $\times 55$

Fig. 51. Second gnathopod, ♀, $\times 55$



(For explanation of above figures see bottom of page 70)

pod 2 in male (fig. 49) much produced into a rounded lobe; in female (fig. 51) produced into a subacute lobe. Palm much longer than hind margin, smooth. Periopods all similar, armed with large sickle-shaped claws, and with fascicles of spines at the joints. The sixth joints have spines arranged in fascicles placed equidistantly along both front and hind margins. Second joints of all pereopods similar, three times as long as wide. Sides parallel in first and second, widening slightly distally in 3, 4, and 5.

Inner plates of maxillipeds armed with 6-8 spines at apex. Outer plates fairly large, reaching about three-fourths the length of the second joint of the palp, armed on inner margin with eight short spines, and at the apex with six longer, more slender spines. Inner plate of first maxilla rudimentary. Outer plate armed with eleven spines at apex. Palp 1-jointed, armed with twelve spines arranged in two rows. Inner plate of second maxilla four-fifths as long as outer, sparsely armed on its inner margin. Cutting edge of mandible multidentate. Spine row with three heavy plumose spines and one smaller one. Molar well developed. Palp extremely large and heavily armed with long curved setae. Second joint the longest. Inner lobes of lower lip well developed. Upper lip slightly incised medially.

First and second uropods biramous. Outer ramus of first four-fifths as long as inner, armed with two rows of spines. Outer ramus only slightly longer than peduncle. Inner ramus of second uropod only slightly longer than outer. Outer ramus half again as long as peduncle. Telson entire, rounded at apex and excavate beneath, surmounted at apex with six strong spines. The leaflike rudiments of the third uropods adhere to its lateral ventral surfaces.

Length 4-6 mm. Antennae, head, and first four segments of body dull brick red; the rest of the body yellowish white.

Remarks.—This species resembles *P. brasiliensis* (Dana) and *P. laevis* (Haswell) in many respects, but differs from them in the production of the fourth joint of gnathopod 2 in the male into a large lobe.

No previous records of this genus have been encountered for the Pacific Coast, *Podocerus californicus* Boeck having been referred to *Jassa* by Stebbing (1906).

Two males and two females found domicolous in an encrusting sponge pulled up from two fathoms.

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EXPERIMENTAL MODIFICATIONS OF
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ON PROTOPLASM

I. AMOEBA PROTEUS

BY

W. A. BLACK

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INTRODUCTION

THIS PAPER presents a study of the effect of ultraviolet radiations on amebas immersed in various salt solutions. It was suggested by the work of Heilbrunn (1932) and Heilbrunn and Daugherty (1933), who suppose that a change in the ionic environment of *Amoeba* may alter the composition of the internal milieu and thereby fundamentally alter the effects of irradiation. *Amoeba proteus*, the animal selected for the present paper, was one of the species used by these authors.

MATERIALS AND METHODS

Animals.—*Amoeba proteus* was obtained from Powers & Powers, of Lincoln, Nebraska. Mast (1926) has given a detailed description of the finer structure of this ameba, and his concepts and terminology have in the main found general acceptance. They will be used here. The organisms were grown in the culture medium prescribed by Chalkley (1930) :

NaCl	0.1000 gm.
CaCl ₂	0.0060 gm.
KCl	0.0040 gm.
Distilled H ₂ O	1000.0 cc.

This stock culture medium is referred to hereafter as "standard medium," or abbreviated as "S. M."

The cultures were set up by pouring this solution into stender dishes to a depth of about 1 cm. and adding about five grains of polished rice and a pipette load of the stock culture, which contained amebas and *Chilomonas paramecium*. The *Chilomonas* served as food for the amebas, which were in good condition for experimentation after about two weeks, and remained so for the duration of the experiments.

Immersion fluids.—Various kinds of dilute solution were used as immersion fluids. These were mainly solutions of the chlorides of Na, K, Mg, or Ca, or mixtures of these, and were either dissolved in water or added to the standard medium. The chemicals used were of the best available reagent grade. The distilled water was supplied by the Department of Chemistry of this University. It was boiled to drive off any CO₂ it may have contained and was kept in sealed containers until used. The solutions were kept in either pyrex glass or wax-

* This paper is based upon research done under the direction of Professor S. C. Brooks in partial fulfillment of the requirements for the degree of Master of Arts in the University of California.

coated bottles. Just before use the pH of all the solutions was adjusted to about 7.0 (the pH of the cultures) by the addition of traces of NaOH. The osmotic pressures of a number of the solutions were calculated and adjusted from the molal freezing-point lowerings as given in "International Critical Tables."

Radiation source.—A "Cold Quartz"* orificial lamp was used for all the experiments reported in this paper. In this lamp a silent discharge is pro-

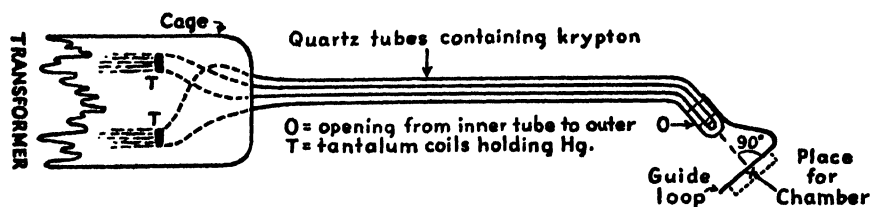


Fig. 1. Schematic diagram of a "Cold Quartz" orificial lamp. The arc is made from one T through O to the other T. Radiations are delivered perpendicularly from all quartz surfaces outside the case.

duced in a mixture of krypton and mercury vapor. The construction of the discharge tube is shown in text figure 1. At least 92 per cent of the radiations of this lamp have been found by Hibben (1931) to be of wave lengths shorter than 2540 Å. Spectrographs of the emission of a lamp of this type are shown in plate 4, figure 1.

Irradiation chambers.—Two types of irradiation chamber were used in the experiments herein reported. They will be referred to as types "A" and "B," and are shown in text figures 2a and 2b. Type "A" chambers were made by cementing an inner glass chamber, consisting of a glass disk supported by three legs, to a coverslip, which was then inverted into an ordinary hollow-ground slide. The height of the inner chamber was 1 mm. In use a drop of the medium containing the amebas was put into the inner chamber so that it was in contact with both the coverslip and the bottom of the inner chamber. Then a little of the medium was placed between the inner chamber and the bottom of the depression so as to minimize changes in concentration of the portion containing the amebas. The construction of this chamber made it possible to include an adequate amount of air and prevent fogging of optical surfaces. It was used in most of the experiments.

Type "B" chambers were essentially depression slides. They were made by boring a hole in an ordinary microscope slide 0.98 mm. thick which was then cemented to another ordinary microscope slide. A drop of the medium containing the amebas was put into the depression and a coverslip was cemented on with "salvoline." The thickness of the film containing the amebas was again 1 mm.

Optical quality fused quartz coverslips less than 0.5 mm. thick were used for the chambers in which amebas were to be irradiated, and ordinary glass disks 1.5 to 2 mm. thick were used as coverslips on the controls. Spectrographs

* Manufactured by the Electro-Therapy Products Corporation, Ltd.

showing their transmittance of the radiations used are given in plate 4, figures 2 and 3.

The chambers were irradiated at a distance of 9 mm. from the end of the lamp discharge tube and in direct line with its axis; this distance was maintained by means of a wire guide fastened to the lamp. The thickness of the coverslip and liquid film between the irradiating source and the amebas made

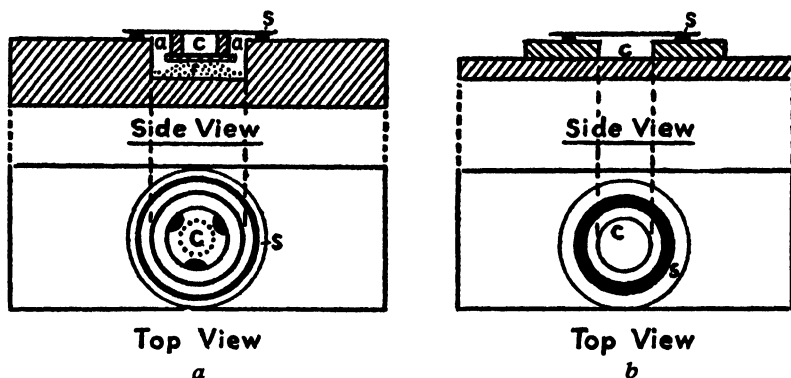


Fig. 2. a. Diagram of the irradiation chamber used in Series I and II. b. Diagram of the irradiation chamber used in Series III.

Legend: a, air space; c, place for the culture; f, fluid to saturate the chamber air; s, salvoline seal.

the distance between the source and the amebas approximately 10 mm. At this distance the intensity was of the order of magnitude of $10,000 \text{ ergs cm.}^{-2} \text{ sec.}^{-1}$.

PRECAUTIONS AND CONTROL EXPERIMENTS

Many minute details of procedure are essential to success in these experiments. Only a few of the more important ones are discussed here; full details are given by Black (1934).

Uniformity and normality of the amebas.—Individuals and cultures of *Amoeba proteus* vary greatly from time to time in form, number of nuclei, nuclear-cytoplasmic ratio, hyalinization, gel-sol ratio, reproductive rate, and activity. These are discussed by Schwitalla (1924), Taylor (1924), Parsons (1926), and Chalkley (1930, 1931). In order to obtain greater comparability all the experiments of a given series were done with a single culture, and all the principal experiments were done with material from two sister cultures.

To avoid unnecessary disturbance in handling the amebas all transfers were made with wax-coated glass capillary pipettes, to which the animals do not adhere.

After the irradiation chambers were loaded with amebas an interval of eighteen hours was allowed to pass, so that they might recover from the disturbance occasioned by the transfer.

The medium best suited to maintain normal appearance, locomotion, and survival of amebas seems to be a very dilute salt solution balanced with respect to the cations Na, K, Mg, and Ca, and having a pH between 6 and 8. Chalk-

ley's standard medium is such a solution, except that Mg is omitted. It seems to be true, however, that distilled water and very dilute solutions of neutral salts of Na, K, Mg, or Ca are almost, if not quite, as favorable (Edwards, 1924; Mast, 1927, 1928, 1931; Hopkins, 1928, 1929). Marine amebas, studied by Pantin (1926), appear to differ from fresh-water amebas in this respect since solutions of appreciably greater concentration are required to maintain the normal osmotic relations, and such solutions of single salts are often toxic to both marine and fresh-water amebas (Reznikoff and Chambers, 1925). Antagonism between the different salts occurs, so that properly balanced solutions of moderate concentration—for example, isosmotic with 0.14 M NaCl—are practically nontoxic even for fresh-water amebas (Reznikoff and Chambers, 1925; Chambers and Reznikoff, 1926; Pollak, 1928).

The basis for comparison within different series of experiments was therefore the group of amebas in standard medium (Series I and III) or distilled water (Series II).

Temperature effects.—The temperature optimum for *A. proteus* seems to be about 23° to 24° C., yet the animals appear physiologically normal at all temperatures from about 11° to 30° C. Even more extreme temperatures are compatible with viability and reproduction (Schwitalla, 1925; Mast and Prosser, 1932; Daniel and Chalkley, 1932).

Experiments were performed to determine (1) whether or not the temperature within the irradiation chamber varied during the exposures, and if so how much, and (2) whether or not the amebas reacted to ultraviolet light differently at one temperature from the way they did at another.

With exposures for lengths of time far greater than the irradiation periods used in the experiments reported in this paper, the temperature variations within the irradiation chambers were less than 1° C. This means that any effects within the irradiation chamber resulting from temperature variation are entirely negligible.

In parallel experiments run at about 10° C. and about 20° C. the differences in results were so slight that they could not be detected with any certainty. Since representative numbers of amebas (20 to 41) were used in all setups, and since the temperature difference (10°) greatly exceeded the fluctuations during irradiation, it is certain that fluctuations in temperature had no perceptible effect upon the results reported in this paper.

Effect of ultraviolet radiations on the medium.—An irradiation chamber was so devised that one-half of the amebas in a continuous medium were protected from the ultraviolet part of the incident light while the other half were exposed to it. The activity of the protected amebas appeared to be unimpaired up to and beyond the time of death of the exposed ones. This shows that the effects of the ultraviolet light are attributable primarily to its direct action upon the amebas and not to an effect upon the medium which is then toxic to them.

Effects of culture fluid.—In the ordinary course of transferring amebas from the cultures to the irradiation chambers a certain amount of salts, or-

ganic solutes, and *débris* would be carried into the fluid in which the amebas were to live and be irradiated. For the purpose of studying the possible influence of these factors the principal experiments were divided into three series. These were as follows:

I. Unwashed amebas were irradiated in solutions of various special salts dissolved in the standard medium (balanced salt solution). This set of solutions provided for the increase and decrease of particular ions in an otherwise salt-balanced medium, containing also traces of organic solutes and *débris*.

II. Amebas were washed and mounted in distilled H_2O solutions of various salts and lactic acid. This set of solutions gave opportunity to investigate the effects of single salts alone.

III. Amebas were washed and placed in solutions like those used in (I) above. Provision was thereby made for studying amebas in the solutions of Series I but with organic solutes and culture *débris* removed.

THE EFFECTS OF INTERMITTENT ULTRAVIOLET IRRADIATIONS UPON AMEBAS IMMERSED IN VARIOUS SOLUTIONS

As a result of the above-described and other preliminary tests designed to establish the nature of the effects to be expected, and the type of experiments best adapted to show those effects, the three series of experiments were done with intermittent ultraviolet irradiations. All the experiments were performed with normal-appearing, mononuclear, adult amebas taken from cultures which were in active growth.

SERIES I. UNWASHED AMEBAS IN STANDARD MEDIUM SOLUTIONS

Method.—Type “A” chambers were filled from a mixture of 1 drop of culture containing several amebas with 4 drops of the special solution being used.

The room temperature during irradiation stayed between 21°5 and 23°2 C. The following solutions were used:

1. Culture only
2. Standard medium
3. Standard medium made isosmotic with solutions 7–9 below
4. NaCl + KCl in standard medium ratio and isosmotic with standard medium
5. Lactic acid-sodium lactate mixture approximately 0.0005M in standard medium, pH = 7.05
6. Same as no. 5 except that it was approximately 0.005M and the pH = 7.3
7. NaCl 0.014M in standard medium
8. $MgCl_2$ 0.010M in standard medium
9. $CaCl_2$ 0.010M in standard medium

The pH of all except no. 5 and no. 6 was maintained at between 6.8 and 7.0 by means of NaOH, and irradiation did not noticeably change this.

Ten irradiations were given at 10-minute intervals, 5-second exposures being used in one set and 10-second exposures in another, in all experiments. In 2 experiments a set of 20-second exposures was added. In each experiment 2 controls and 2 experimental mounts were used. One of the controls was kept in the dark and labeled "DC" and the other was irradiated with the experimental ones but protected by a glass guard and labeled "IC." The experimental chambers were given numerical labels—for example, "5," "10"—which indicated the length of the individual exposures given.

The amebas in the chambers were examined just before, during, within 1 hour after, and 24 hours after the last irradiation. Observations were made with respect to:

- (1) Activity following irradiation
- (2) Cytolysis following irradiation
- (3) Form and general appearance following irradiation

Data and results.—Most productive of data were the degree of activity and the degree of cytolysis among the irradiated amebas. Two degrees of activity may easily be distinguished, namely *active*, which refers to all individuals showing progressive locomotion, and *quiet*, which refers to all viable individuals which are sessile or show only sluggish random movements. Examination of irradiated amebas seemed to indicate a sequence of morphological conditions from normality to cytolysis. Consequently, records were made of these forms with the hope of establishing a morphological lethal threshold for the irradiations. These forms were common to all three series of experiments and are described in detail after consideration of all the data on activity and frequency of cytolysis. It was ultimately found that recovery from any stage short of actual cytolysis might occur. The data concerning activity and cytolysis are given in graphic form in text figure 3. Cytolysis is here used as synonymous with death of the organism.

This series of experiments shows that:

(1) The solutions alone without irradiation affected the motility of the amebas, which remained fully active only in the standard medium, the pure culture medium, and the aqueous solution of NaCl + KCl, all of which are isosmotic with one another. All the solutions of higher osmotic pressure decreased appreciably the activity of the amebas, and the more dilute lactic acid-lactate solution was intermediate.

(2) Irradiation produced changes which gradually decreased the activity of the amebas in all the solutions, but the difference in activity between irradiated and control groups does not appear to be materially affected by the composition of the immersion fluid. The lactate mixtures are the only solutions showing any suggestion of conserving the activity of *irradiated* amebas.

(3) Cytolysis occurred to a slight degree in all the control experiments except when the standard medium or its more concentrated form was used.

(4) Irradiation increased the degree of cytolysis, two minor exceptions lying within experimental error.

(5) The three solutions which maintained the *unirradiated* amebas in the

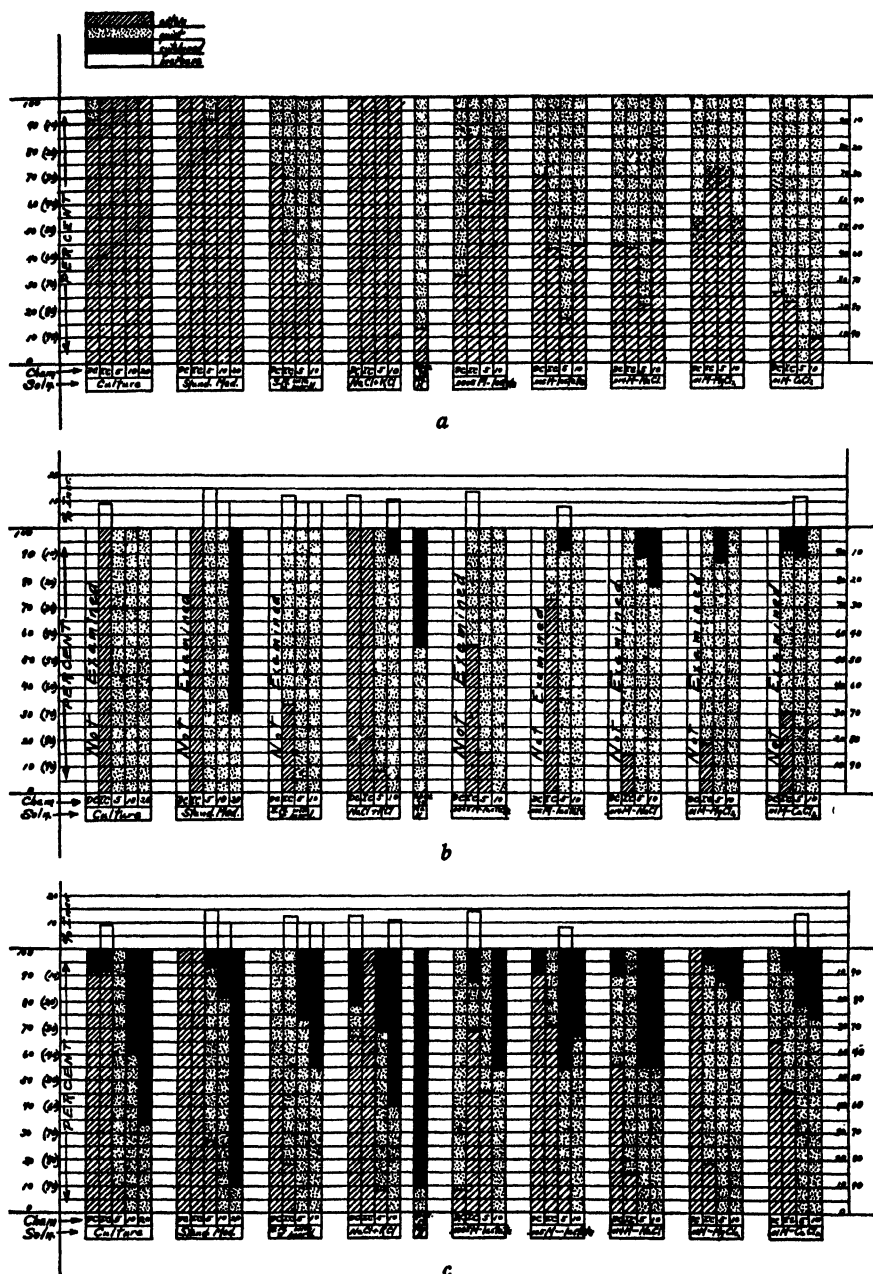


Fig. 3. Chart showing for Series I the relative amounts of active, quiet, and lysed amoebas in the various chambers and solutions before and after irradiation. *a*. Just before irradiation. *b*. One hour after irradiation. *c*. Twenty-four hours after irradiation.

most active state showed the greatest increases of cytolysis as a result of irradiation. Those which tended to induce quiescence protected the amebas against irradiation. (This relationship is not maintained in the other two series.)

(6) This protective effect is particularly pronounced with respect to the solutions enriched with Ca and Mg. The lactic acid-lactate solutions have some protective power, being about as effective as the concentrated standard medium. This result is surprising in view of their tendency to reduce the effect of irradiation on motility.

SERIES II. WASHED AMEBAS IN SINGLE SALT SOLUTIONS

Method.—A series of tests similar to those described above was done upon washed amebas immersed in distilled water and distilled water solutions of various salts and lactic acid. The amebas were washed by transfer into three successive changes of the appropriate solution and then into the same solution in the type "A" exposure chamber.

The solutions used were:

1. Distilled H_2O pH = 6.6
2. 0.0005M lactic acid-sodium lactate mixture pH = 7.2
3. 0.001M-KCl pH = 6.7
4. 0.001M-NaCl pH = 6.7
5. 0.001M-MgCl₂ pH = 6.6
6. 0.001M-CaCl₂ pH = 6.75

Irradiations of 5, 10, 15, and 20 seconds were given 10 times at 10-minute intervals. No "dark" controls (DC) were included in this series of experiments. The results of the previous series indicated that the "irradiated" controls (IC) were sufficient. The temperature during irradiation was between 21°3 and 22°7 C.

It should be noted that the osmotic pressure of these solutions is considerably less than that of those used in the experiments of the previous section. They correspond to 0.001M salt solutions in distilled H_2O as compared with 0.010M-0.014M in standard medium.

With the exception of the above-mentioned variations the details of procedure for this series were the same as for the preceding one.

Data and results.—Since the nature and purpose of this series of experiments were the same as those of the previous one the data will be presented without further introduction (text figure 4). The terms used have the same meanings as before.

These data show that:

(1) The differences in activity supported by the various solutions are probably not significant except with respect to the lactic acid-lactate and possibly the CaCl₂ solutions. The lactic acid-lactate definitely inhibits the activity of unirradiated amebas.

(2) Both immediately after and 24 hours after irradiation the amebas in all the chambers except those of the 5-second irradiation series were quiescent.

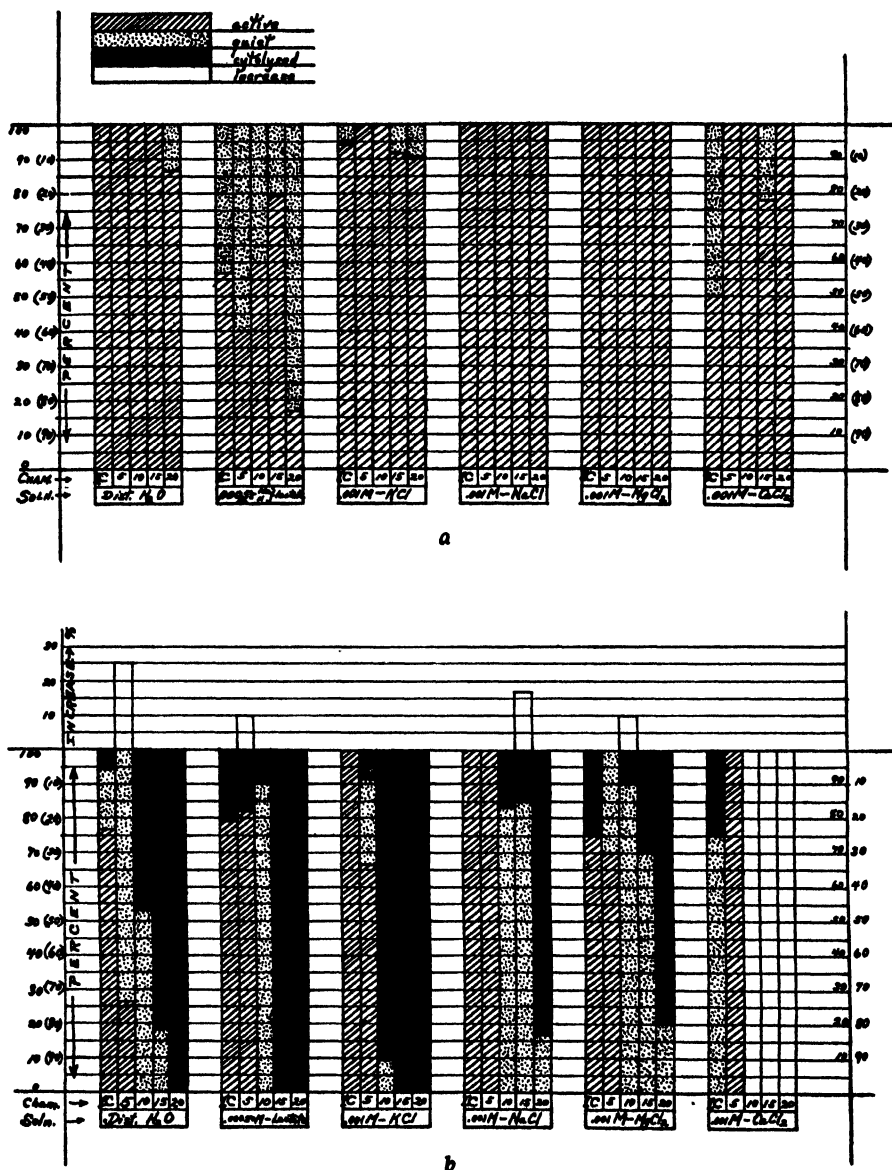


Fig. 4. Chart showing for Series II the relative amounts of active, quiet, and cytolysed amoebas in the various chambers and solutions before and after irradiation. a. Just before irradiation. b. Twenty-four hours after irradiation.

(3) Twenty-four hours after irradiation the amebas that were given ten 5-second exposures were least active in H_2O and most active in the $NaCl$ and $CaCl_2$ solutions, but the differences are not great. Na and Ca seem to conserve the activity of *irradiated* amebas.

(4) Twenty-four hours after irradiation the degree of cytolysis in the various solutions appeared to be in the following order: $Ca < Mg$, $Na < \text{distilled } H_2O$, lactic acid-lactate $< KCl$. It may be noted that the unirradiated amebas in KCl were all in an active condition, while 91 per cent of those that were given ten 10-second exposures were cytolysed. In contrast to this 21 per cent of the unirradiated amebas in the lactate mixture were cytolysed, but only 10 per cent of those that were given ten 10-second exposures. Attention may also be directed to the fact that for the $CaCl_2$ solution definite data were available only for the control and 5-second irradiation chambers.

SERIES III. WASHED AMEBAS IN STANDARD MEDIUM SOLUTIONS

Method.—Type "B" chambers were loaded with amebas from the same culture as was used for the experiments of Series I.* They had previously been washed in three successive changes of the appropriate solution. The solutions were:

1. Standard medium unaltered
2. Standard medium isosmotic with solutions 5–7 below
3. $NaCl + KCl$ in standard medium proportions and in solution isosmotic with solution 1 above
4. Lactic acid-sodium lactate mixture, approximately 0.005M in standard medium, $pH = 6.8$
5. $NaCl$, 0.014M in standard medium
6. $MgCl_2$, 0.010M in standard medium
7. $CaCl_2$, 0.010M in standard medium

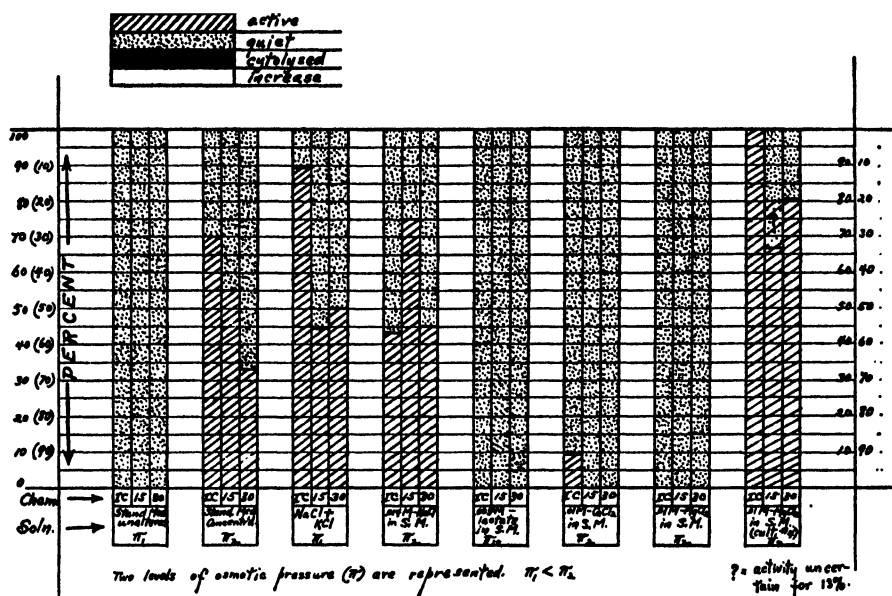
The pH of all was maintained at from 6.8 to 7.0 by the addition of $NaOH$ when necessary.

In this series of experiments only 3 chambers were used for each solution: 1 irradiated control, and 2 irradiated experimental chambers. The method of irradiation was the same as that used in the two previous series, but the exposures differed. One experimental chamber received a 15-second exposure 5 times at 8-minute intervals: the other received a 30-second exposure 5 times at 8-minute intervals. The room temperature during the irradiations in this series of experiments varied between 21.6 and 23.2 C. Observations were made just before, just after, and about 40 hours after irradiation.

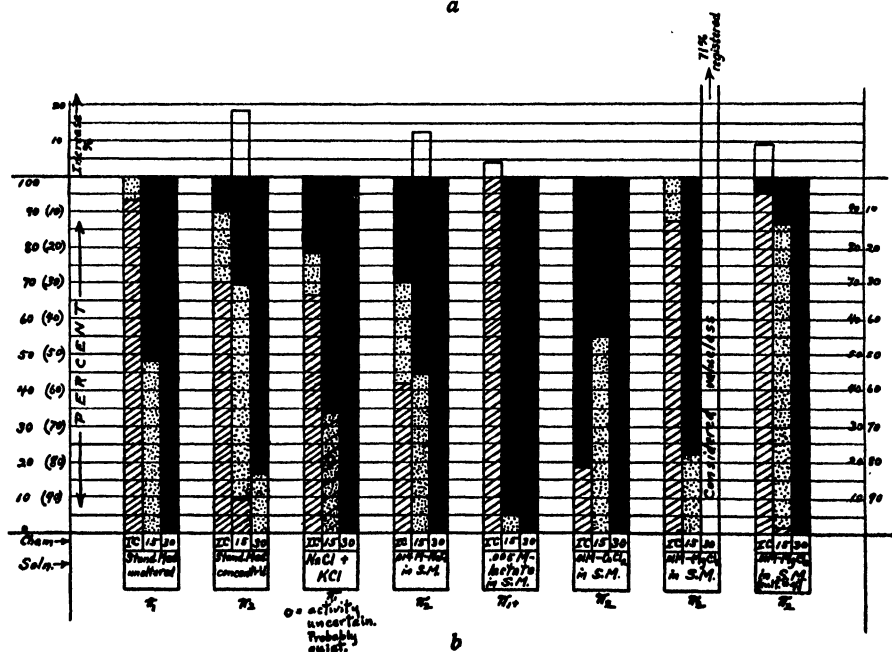
Results.—The effects of this treatment on activity and cytolysis are represented in text figure 5. The findings may be summarized as follows:

- (1) The solutions were found to effect the activity in the following manner: the standard medium, lactic acid-lactate, $MgCl_2$, and $CaCl_2$ solutions induced quiescence, and the concentrated standard medium, $NaCl$, and $NaCl + KCl$ solutions maintained activity.

* It was found necessary to make a second preparation for the $MgCl_2$ solution, and for this another culture was used.



a



b

Fig. 5. Chart showing for Series III the relative amounts of active, quiet, and cytolysed amoebas in the various chambers and solutions before and after irradiation. a. Just before irradiation. b. Forty hours after irradiation.

(2) Irradiation abolished activity except in the concentrated standard medium, which maintained 9 per cent activity in the 15-second irradiation chamber. The $MgCl_2$ solution is not comparable with the others, because a different culture of amebas was used.

(3) Just after irradiation, the solution having an increased amount of Ca was most noticeable in the protection which it afforded against cytolysis, and the lactate solution was most noticeably damaging.

(4) Cytolysis among the unirradiated controls of the solutions used occurred in the following order: $CaCl_2 > NaCl > NaCl + KCl > \text{concentrated standard medium} > \text{all other solutions}$ (which show no cytolysis).

(5) Similar comparison within the 15-second exposure series shows the degrees of cytolysis to be: lactic acid-lactate $> MgCl_2 > \text{standard medium}$, $NaCl + KCl > NaCl$, concentrated standard medium $> CaCl_2$.

(6) Among the experiments of the 30-second exposure series the only one showing viable amebas 40 hours after irradiation was the concentrated standard medium. Consequently, comparisons are not possible.

PROGRESSIVE CHANGES OF FORM INDUCED BY IRRADIATION

In Series I and II the animals in the irradiation chambers were specially observed from time to time during and after the irradiations, so that it might be determined whether the ultraviolet irradiation produced definite and characteristic morphological changes. It was hoped that it might be possible to classify the form and general cytoplasmic appearance of the amebas according as they were or were not fatally injured.

Various stages of the visible effects of ultraviolet irradiation are illustrated by the photomicrographs in plate 5, figures 1-9. Omitting some unimportant variants, these stages may be distinguished as follows:

(1) Stoppage of the active pseudopod.

(2) Retraction of the pseudopod. This leaves crenulations in the membrane.

(3) Sometimes there occur second attempts at pseudopod formation and retraction, or merely slight uncoördinated back-and-forth protoplasmic streaming.

(4) Rounding.

(5) The next stage was characterized by the assumption of a definite form clearly indicative of injury. This is a more or less rounded form, bloated and often with hyaline areas. Pseudopods, if any are present, are very broad and blunt and are not clearly distinguishable from hyaline blisters or blebs which arise on the surface of the ameba. The latter are usually more clear and are presumably filled with a clear fluid. Apparently the first bleb arises on the part of the surface which was least active at the time pseudopod formation was stopped. Usually this is directly opposite the last pseudopod.

(6) In some of the animals observed there was a tendency to become spherical with a hyalinized periphery, that is, with the granules clumped more or less in the central part or near the site of the last pseudopod. During this stage and especially the preceding one there frequently occurred a sudden smooth-

ing out of the indentations between adjacent lobes or a sudden bulging in the surface with a localized flow of granules into the region filled out. It appeared as if there were a local liquefaction which destroyed the internal structural restraints. The crenulations and "button" which follow the retraction of the last pseudopod are very persistent.

(7) Rupture, or coagulation. Ruptures may occur simultaneously at different points or only a single rupture may occur. Ruptures sometimes heal immediately. The brownish coagulum which appears in the hyaline matrix of amebas immersed in CaCl_2 solutions and cytolysed by ultraviolet light seems to appear quickly at the time of cytolysis. Up to this time Brownian movement and migration of granules indicate fluidity.

All these conditions, however, look like those brought about by other toxic agents. Although in general the bloated forms (5) lead to cytolysis, recovery was noticed in a large number of animals. The globular, highly hyalinized form (6) from which recovery was never found to occur was only an occasional sequel of irradiation. No regularly occurring living form marking the stage from which recovery is impossible, was found; hence no definite morphological criterion with respect to the degree of injury could be established, and it seems to be impossible to predict accurately upon the basis of morphological appearance whether or not cytolysis will occur. The hypothesis might be made that the bloated form found to be indicative of injury is only a symptom of rather generalized cytoplasmic impairment, and that recovery does or does not occur according as some other invisible but vital entity has or has not been destroyed. However this may be, cytolysis usually follows whenever an ameba has definitely assumed the bloated form.

EFFECTS OF IONIC ENVIRONMENT ON THE TYPE OF CYTOLYSIS

The way in which cytolysis resulting from ultraviolet irradiation occurred in different solutions was rather characteristic. Cytolysis, as the term is used here, involves three types of process: membrane rupture, membrane disintegration, and change in the appearance and consistency of the cytoplasm. The first two may be different expressions of the same process.

In all animals except those in solutions having an excess of CaCl_2 , cytolysis following irradiation occurred by membrane rupture or disintegration and cytoplasmic effusion. It was in general preceded by a distinct increase in hyalinization, and at no stage was Brownian movement in the cytoplasm seen to stop. When the membrane ruptured, part of the fluid contents of the amebas was forced out, and following this the membrane appeared to shrink. The membrane was left as a partly empty shell, which disintegrated with time, but at a rate which seemed also to be affected by the ionic environment. The three principal types of cytolysis are shown in plate 5, figures 10-12. Special characteristics of cytolysis appearing in some of the different solutions were observed as follows:

Distilled H_2O .—Cytolysis was characterized by wide dissemination of the cytoplasm upon rupture of the membrane. There appeared to be an elastic

contraction of the ruptured membrane which resulted in forcible expulsion of the contents of the cell. The membranes were destroyed in a relatively short time.

The lactate solution.—Cytolysis occurred with a general disintegration of the membrane as opposed to simple rupture, and with incomplete effusion of the cytoplasm. The cytoplasm seemed freely miscible with the surrounding medium, but the general breakdown of the membrane left no elastic structure which could expel more of the cell contents.

0.001M-KCl.—Cytolysis appeared with extensive breakdown of the membrane and wide dissemination of the cytoplasm. Complete destruction of the membranes occurred in a relatively short time.

0.001M-NaCl.—Cytolysis occurred with membrane rupture and moderate effusion; complete membrane destruction occurred fairly early.

0.001M-MgCl₂.—Cytolysis occurred with membrane rupture and a small amount of effusion. Membrane destruction was much slower than in the solutions previously mentioned. The granules remaining with the membrane kept well clumped together.

0.001M-CaCl₂.—Cytolysis characteristically occurred without rupture. Brownian movement stopped at the moment of death, and the cytoplasm was converted into what appeared to be a solid, mottled, brownish coagulum. After some time the membrane disintegrated.

0.010M-CaCl₂ in standard medium.—Cytolysis following irradiation appeared as a "coagulation" with stoppage of Brownian movement, resulting in a rigid structure brownish in color and presenting a flaky matrix. This seemed to arise from the hyaline material. Ruptures sometimes occurred, but they immediately healed, so that a general outflow was prevented. Clumped cytoplasmic masses were left in the solution after complete membrane destruction.

DISCUSSION

Under the conditions of these experiments amebas lived normally or with at most a slight decrease in active motility as long as observed (i.e., 58 hours) in the standard medium, which was a very dilute physiologically balanced salt solution. The other immersion fluids often led to more or less complete quiescence, and occasionally to the cytolysis of a small number of the amebas present.

The effects of ionic surroundings and of handling can best be judged from the condition of all the amebas just prior to irradiation, that is, 18 hours after transfer to the solutions. The samples here number about 50 animals, whereas thereafter only the controls, numbering about 10–20 animals, are available for study of these effects uncomplicated by irradiation.

The contrast between Series III and I, done with animals all taken from a single culture, shows that either handling or elimination of culture-medium constituents reduces the activity of the amebas, the mean percentage of active forms in comparable solutions being: unwashed, 59; washed, 25. Similar differences appear 24 hours later, including an appreciably greater cytolysis

among washed amebas (24 per cent) as compared with unwashed (4 per cent). Because of the difference in cultures and in osmotic pressure and composition of solutions, the contrast between Series II and Series I cannot be used as evidence of the effects of washing. Although washing appears to have increased the differences between different solutions in Series III, this is not true of Series II. On the whole it is probably advantageous to take this precaution, since injury is at the most quite small, and at the same time undesirable unknown variables are eliminated.

There is some evidence in all series that an increase in osmotic pressure, such as that between standard medium and concentrated standard medium, favors quiescence. In general, activity is less in the more concentrated media of Series I and III than in Series II. However, Series II, lacking the physiologically favorable mixture of the ions of the standard medium, is again not comparable.

The different series show little agreement with respect to the possible causes of induced quiescence or continued activity of unirradiated amebas, and all that can be said with any assurance is that the lactic acid-lactate mixtures led typically to a condition of considerable quiescence, and that H_2O and solutions lacking bivalent ions such as the $NaCl + KCl$ mixture induced considerable activity. Solutions containing only small concentrations of Ca and Mg did not seriously reduce activity, although the reduction in the Ca solution may be significant (Series II). Solutions 0.010M with respect to Mg and Ca , especially the latter, evidently inhibit activity (Series I and III). This would tend to confirm the conclusions of many authors (Edwards, 1923, 1924; Hopkins, 1929) that motility is affected by the proportions in which different ions are present in the immersion fluid. The lactic acid-lactate mixtures might be expected to favor penetration of lactic acid into the amebas by membrane hydrolysis, thus decreasing their internal pH. If this occurred it might lead to viscosity changes in the cytoplasm (Barth, 1929) or to changes in the sol/gel ratio. This in turn would constitute an alteration in the structural mechanism of movement, and would thus account for the observed decrease in motility. Too little is known about the intermediate changes to permit closer analysis.

Loss of motility, change of form, and cytolysis are three pronounced phenomena which appear upon irradiation. The first has been described above. The changes in form were not obviously affected by the nature of the immersion fluid, nor even, as has been pointed out above, peculiar to irradiated amebas. Careful study showed that there is no fixed distinction between "reversible" and "irreversible" changes in appearance. Form is therefore an unreliable guide to the extent of the injury resulting from ultraviolet irradiation. Cytolysis, on the other hand, furnishes an end point from which recovery is impossible, and it was found to afford the clearest evidence with respect to the effects of the immersion fluids on the degree of the injury resulting from irradiation. Furthermore, various types of cytolysis were characteristic of the different fluids, and have been described above.

Observations on the viscosity of ultraviolet irradiated eggs of *Arbacia*

punctulata led Heilbrunn and Young (1930) to a hypothesis according to which an important secondary effect of the irradiation was supposed to be the liberation of free Ca^{++} in the egg cytoplasm. Similar viscosity changes were observed in *Amoeba dubia* and *Amoeba proteus* by Heilbrunn and Daugherty (1933), and general validity has been claimed for the hypothesis. Increase in free Ca^{++} concentration is supposed to lead first to a transitory decrease in viscosity, such as had been observed, and then to a specific protoplasmic reaction, analogous to blood coagulation and, like it, dependent upon the presence of free Ca^{++} , and resulting in the formation of insoluble material in the form of a coherent mass or clot. This is the type of change often called "coagulation" by analogy with blood "coagulation," though it is probably not a true coagulation in the sense in which that term is employed in protein chemistry.

According to this hypothesis it would be predicted that the absence of Ca from the immersion fluid should be protective, if anything, and conversely that the addition of Ca to the immersion fluid should, if anything, aggravate the injury done by light. If such modifications of the standard medium were found to be without effect, this might be attributed to impermeability of the cells to Ca. But in the present experiments the opposite effect was produced. In all three series the percentage of surviving uncytolysed amebas was on the average greater after irradiation in the CaCl_2 solutions than in any other solutions, in spite of the fact that unirradiated controls showed about the same or greater cytolysis (Series III) in these solutions than in the others. Increase in Ca^{++} content of the immersion fluid was therefore protective against killing by ultraviolet light. MgCl_2 solution was abnormal. This result is in better agreement with Tehahotine's experiments in which he found that the addition of CaCl_2 to sea water greatly retarded the local cytolysis caused by ultraviolet light. (Tehahotine, 1920). Tehahotine worked with eggs of an unnamed sea urchin, probably *Strongylocentrotus lividus*. Moreover, the observation described above as typical of cytolysis in Ca-rich solutions, that membrane modification seems to precede the "coagulative" change in the cytoplasm, would seem to support the idea that the integrity of a semipermeable surface membrane was the crucial condition for the survival of the ameba, and that, as in Tehahotine's experiments, Ca protects this membrane. Solutions lacking Ca, with the possible exception of NaCl , had on the contrary a tendency to be synergistic with ultraviolet light. The " $\text{Na} + \text{K}$ " solutions in Series I, but not in Series III, show this synergy, and it is exceedingly conspicuous in the pure aqueous KCl in Series II. A possible protective effect of Na might be deduced principally by contrast with pure water or solutions containing K (Series II).

In another paper (Black and Brooks, MS) it will be shown that ammonium salts like the lactic acid-lactate mixtures, are synergistic with ultraviolet irradiation. We may note that the former tend to raise the protoplasmic pH, and the latter to lower it. Although the pH effects of the two media seem opposed, it is noteworthy that Barth (1929) found that in *Arbacia* eggs the viscosity of the cytoplasm was decreased by small concentrations of both acetic acid and ammonium hydroxide. It is possible that the similarity between

lactic acid-lactate solutions and ammonium salts may in the present experiments rest upon a similar resemblance between their effects. We may therefore make the tentative conclusion that solutions which tend to lower protoplasmic viscosity are synergistic with ultraviolet light. All these effects of various ions can still be explained on the basis of Heilbrunn's hypothesis, although this requires some purely *ad hoc* assumptions. These protective and synergistic effects will be more fully considered in connection with other experiments described in another paper.

The manner in which cytolysis occurs in different solutions is consistent with the idea that ultraviolet irradiation destroys the structure of the plasmalemma, so that it finally ruptures. Rupture is probably caused by increased internal pressure, which first shows itself by increase in volume and attendant rounding up of the amebas. At first the volume may increase without greatly stretching the membrane, simply by approach to a spherical form. But when the form of the ameba has become nearly spherical, further swelling must stretch the membrane. Unless this is elastic or has considerable strength, it must ultimately rupture. In the CaCl_2 solutions the membrane appears to lose its semipermeability without rupture so that Ca enters the ameba and Heilbrunn's specific protoplasmic reaction takes place. This results in the formation of a coherent, relatively opaque, mottled, brownish mass. This phenomenon occurs whenever too much Ca is present in the cytoplasm, and is not a result of irradiation as such. When the immersion fluid contains Ca the irradiation seems to make it possible for the Ca to get into the cell, whereas the cell is normally practically impermeable to this ion. Mg has a somewhat similar effect; it does not prevent rupture, yet it does produce something like clot formation in the cytoplasm, and thus retards its dissipation in the surrounding medium. In all the other solutions effusion of cytoplasm through the ruptured membrane is more or less marked, and the protoplasm disperses freely in the surrounding solution. All these again are entirely like the effects of tearing amebas in similar solutions (Reznikoff and Chambers, 1925) and are not characteristic effects of irradiation.

The most important effects of ultraviolet irradiation of amebas seem therefore to be (1) interference with motility, (2) swelling, and (3) destruction of the semipermeable surface membrane, which is perhaps the same as, or part of, the plasmalemma. It is quite possibly significant that solutions which favor quiescence of unirradiated amebas tend to preserve their motility when they are irradiated with ultraviolet light, and that the same solutions tend to protect against the cytolytic effects of irradiation. Other solutions tend to increase the motility of unirradiated amebas, but to exaggerate the decrease in motility and increase the cytolysis resulting from irradiation.

There thus appear to be at least two effects of ions in the solution, the first internal and affecting motility and the second an action upon the semipermeable surface membrane, affecting both motility and the integrity of the membrane. Irradiation probably affects both the cytoplasm and the membrane (directly or indirectly); the crucial effect seems to be that on the membrane.

SUMMARY

Amebas (*A. proteus*) were irradiated in dilute media of differing ionic composition, light of wave lengths mostly equal to or less than 2540 Å being used.

The ensuing morphological changes are described and pictured. Except for certain differences in form and motility caused by the ionic environment, the changes are typical of injury in general and are not characteristic for irradiation.

The activity of unirradiated amebas was usually decreased in solutions having relatively large amounts of Ca or Mg, and in lactic acid-lactate mixtures of about the same pH, namely, 7.0. It was usually increased in solutions lacking Ca or Mg, or high in K. A dilute balanced salt medium formed the basis for comparison.

Media which alone favored motility (e.g., KCl) tended to be synergistic with irradiation in decreasing motility and producing cytolysis, and vice versa.

These results support Tehahotine's contention that Ca (and presumably also Mg) oppose the effects of ultraviolet light on the plasma membrane, the breakdown of which leads to cytolysis and death. They do not support Heilbrunn's theory of the cause of death from ultraviolet irradiation, but are not a definite disproof thereof.

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EXPLANATION OF PLATES

PLATE 4

Plate 4. Spectrographs of the radiation from a "Cold Quartz" orificial lamp similar to the one used, and showing the transmittance of the different chamber windows.

Fig. 1. Spectrograph of the radiation from a "Cold Quartz" orificial lamp. (From Hibben, 1931, fig. IIb.)

Fig. 2. *a.* Spectrograph showing the ultraviolet light (from a quartz mercury arc) transmitted by the quartz coverslips used as chamber windows. *b.* Mercury arc wave-length guide.

Fig. 3. *a.* Spectrograph showing the ultraviolet light (from an aluminum arc in hydrogen) transmitted by the glass coverslips used as chamber windows to admit the visible but to screen out the ultraviolet. *b.* Iron arc wave-length guide.

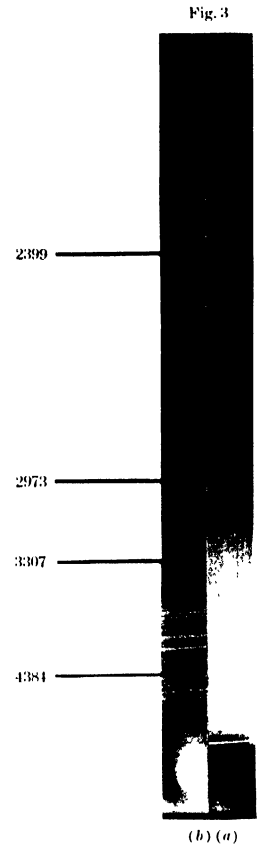
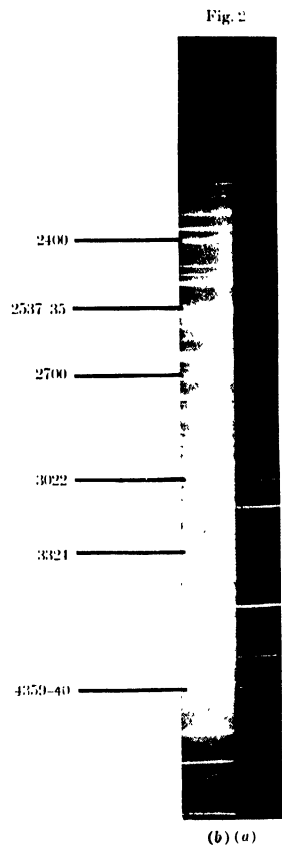


PLATE 5

Plate 5. Photomicrographs of amebas taken at various stages during the experiments. Where different forms occur in a single figure the one to be noted is encircled. Magnification of figs. 1 and 3, $\times 150$; of all others, $\times 120$.

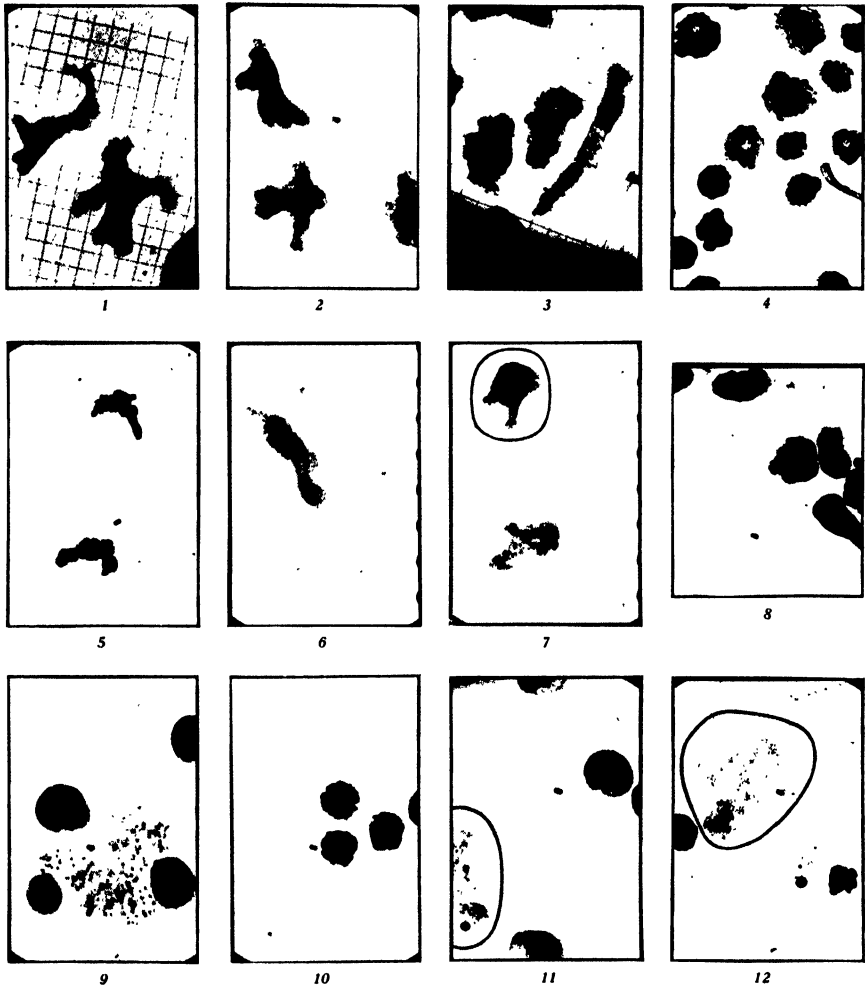
Figs. 1-4. Various normal forms of the amebas.

Figs. 5-9. Progressive changes from the normal state to cytolysis brought about by ultraviolet radiations.

Fig. 10. The typical end form when cytolysis occurs by "coagulation," as in solutions rich in CaCl_2 .

Fig. 11. Cytolysis with incomplete membrane destruction and moderate effusion, such as occurs in the standard medium.

Fig. 12. Cytolysis with general breakdown of the cell membrane and wide dissemination of the cytoplasm, such as occurs in KCl solutions.



APPARATUS FOR THE DISSECTION AND STUDY OF EMBRYOS

BY

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INTRODUCTION

PERHAPS THE MOST DIFFICULT THING encountered by the student of embryology is the attempt to visualize in his mind or on paper the three-dimensional object which he is studying in sections. It is so difficult even for the trained investigator that resort is constantly had to the valuable but time-consuming and tedious method of making reconstructions in wax. In order to display the anatomy of the rat embryo without the use of models, the writer, in 1925, experimented with knives made out of Darwin razor blades, and began devising means for operating them mechanically under the dissecting binocular microscope. Also, after careful study and experimentation, a camera was evolved for photographing dissections stereoscopically.

THE STEREOSCOPIC PHOTOGRAPH AND ITS USE

By means of the stereoscope a pair of flat photographs merge into a picture containing vivid details and startlingly real perspectives. Thus, the exquisite perfection of an embryo is disclosed with clearness, accuracy, and depth in the stereoscopic picture, as when it is seen under the binocular microscope.

One objection to the photographs is that they must be viewed through a more or less cumbersome stereoscope, but the three-dimensional effect can be obtained without its use if the two pictures are separated by no more than the interpupillary distance of the observer. Under these conditions there is no strain to the eyes, for, although ordinarily associated, the lens compensation for distance and the degree of convergence of the eyes are actually independent. It is possible to get the stereoscopic effect with pictures separated by more than the distance between pupils, causing the eyes, with great strain, to diverge. For the average person, the interpupillary distance is about $2\frac{3}{8}$ to $2\frac{1}{2}$ inches (60–64 mm.). The pictures in this paper are spaced $2\frac{1}{4}$ inches apart, making it easy for most readers to get the effect of binocular vision with unaided eyes.

The following suggestions may assist the reader to view the illustrations without a stereoscope. Let him, while holding the pair of photographs at ordinary reading distance, look just above them at some distant object until aware that there seem to be three (or four) images of the photographs. The middle image is a mental synthesis of the two and has the added feature of the third dimension. If there are four images to begin with, the two middle ones must be made to coalesce. When the effect of depth is obtained there will always seem to be three images—the middle one stereoscopic, the side ones flat. When the binocular effect is obtained, the eyes do not look partly "cross-eyed" as when reading; the two lines of vision are nearly parallel and each eye is looking at its own picture. It is essential, with or without a stereoscope, to see that the

pictures are horizontal or, rather, parallel to the line between the pupils. Keeping the pictures within the interpupillary distance of each other necessarily limits their size.

ILLUMINATION

One of the first requisites for dissection is adequate illumination. Since the arc light is troublesome to use, and the small microscope lamps on the market are not satisfactory, the writer has designed a small illuminator, the principle of which is shown in figure 1, and general views of which are shown in plates 6, top, and 8, top.

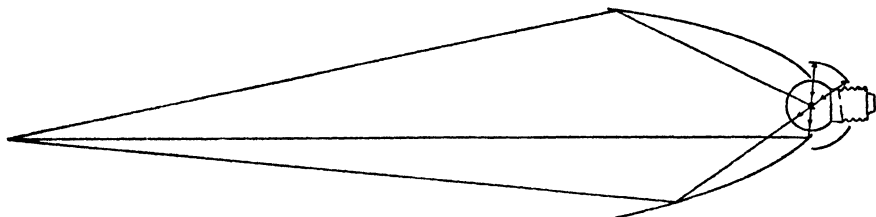


Fig. 1. About $\frac{1}{2}$ natural size. Diagram of flashlight bulb, elliptical reflector, spherical reflector, and the courses of the light rays. When the bulb is properly centered, light falling on the spherical reflector is returned through the bulb and filament to the larger reflector, which concentrates all the light it receives to a point about 7 inches from the front of the lamp.

The illuminator consists essentially of two reflectors, one elliptical and the other hemispherical, by which about 90 per cent of the light from a flashlight bulb is concentrated on a very small area at a distance of 7 inches from the rim of the large reflector. The use of the two reflectors and the small bulb makes possible a long working distance with a much smaller elliptical condenser than would otherwise be required. The course of the light rays is shown in figure 1, wherein it will be seen that light from the basal half of the bulb is returned by the spherical reflector through the filament to the large reflector. Provision is made for centering accurately the lamp filament in the spherical reflector, and for adjusting the two reflectors with respect to each other. Current is supplied by a small bell transformer. The cost of operation is very low.

DISSECTING APPARATUS

The tools for dissecting consist of cutting knives made from Gillette Darwin blades, dissecting needles, fine-tipped forceps, and minute brushes.

The knives, attached to what may be called clips, are made to vibrate rapidly in a direction parallel to their edges by a pair of small magnets actuated by the commercial 110-volt, 60-cycle alternating current. As can be observed in plate 6, top, a glass cover protects the magnets, armature, etc. The armature is attached to a slender rod (pl. 6, bottom, *a*) which extends the whole length of the instrument, protruding at the lower end (pls. 6, bottom, and 7, top). By adjusting the nuts on the upper end of the rod, and at *x* (pl. 6, bottom, *a*) against the springs, the opposing springs are balanced against each other so that the rod (and armature) is suspended between the springs, and, when the current is flowing, is caused to oscillate lengthwise gently to vigorously according to the number of resistance lamps in series with the magnets. Lamps

totaling 10, 20, 30, 40 watts (pl. 6, top) give a sufficient range of power. The lower end of the rod (pl. 6, bottom) transmits motion to the cutting knife either directly, as to a lancet-like knife screwed to the end, or, as more commonly employed, indirectly and transversely by a simple device (clip) to a knife which then cuts in a horizontal plane. This latter type of knife may have any width (length of edge) from .10 to 10 mm. It is made by breaking off a piece of a Darwin blade, holding it in a little steel clamp or small vise, and grinding it to the desired size and shape (pl. 6, bottom, and 6, bottom, *b*). It is then soldered to a strip of phosphor bronze .013 inches thick (28 gauge), and $\frac{5}{16}$ inches wide, which is bent at an angle of about 45 degrees. It is this strip which cuts the surface of the alcohol, and, because it vibrates edgewise through the fluid, produces a minimum of disturbance. This strip is in turn soldered, as is shown in plate 6, bottom, and 6, bottom, *b*, to another bent piece (90°) of bronze, the long arm of which is soldered to a split ring of brass. The "clip," then, consists of these two strips of bronze and the brass ring. The ring of brass, when the knife is in use, is slipped over the brass tube which forms the case of the vibrating rod, until the short arm is in contact with the end of the rod. The degree of pressure exerted by the arm on the rod together with the current employed determines the amplitude (and in part the vigor) of the motion. It may be made strong enough to cut fixed and hardened uterine musculature, and placenta, or slight enough to enable one to cut thin membranes or to work in narrow spaces. As an aid to making a delicate adjustment, the brass casing above the clip may be changed in length by means of the milled collar with fine threads (pls. 6, bottom, and 7, top). With a sharp blade properly used, slices of tissue a few micra thick can be shaved off in dissecting.

As shown in plate 6, top and bottom, the vibrator is slipped into a spring clamp attached to a mechanical stage so adapted that, by means of the racks and pinions, the dissecting instrument can be moved vertically and in one horizontal direction with precision. The second horizontal direction is only very occasionally needed and can be supplied by moving the dish containing the embryo. In fact, it frequently is more desirable to keep the vibrating knife in the center of the field and to move the embryo by sliding its container on the turntable with the left hand. Thus, when one wishes to finish a cut with an upward movement, the right hand is left free for operating the vertical rack. Delicately controlled vertical movement is facilitated by the detachable arm (pl. 6, top).

The mechanical stage is supported on a vertical post by a simple clamp which allows both vertical and horizontal adjustment. The post sits in a socket at the end of a flat arm the other end of which, about the center line of the microscope, is firmly held on the baseboard by a screw which permits the arm to be swung to one side.

For grinding knives a small, fine carborundum wheel, motor-operated, is set up in a horizontal position under a binocular. It is essential that grinding be controlled under a microscope. This is especially true in the grinding of dissecting needles and fine-tipped forceps. Indeed, for the former, it has been necessary to set up a special tapered spindle to which a hypodermic needle

can be fitted, and which can be tilted at any angle and moved by means of screws.

The dissecting needle, operated by means of a holder to be described presently, is best made from a hypodermic needle for the simple reason that alcohol can be squirted through it to wash away loosened bits of tissue. By means of the grinding apparatus a part of the wall of a needle can be transformed into a slender filament of steel with a point 10 to 20 micra thick. The tip can be used straight or bent as desired.

The needle holder (pl. 7, top) is made on the principle of the pantograph. The needle fits on the lower end of a piece of small brass tubing the upper end of which is closed and fitted with a small steel ball which slides snugly through a short, larger tube. This steel ball supports the upper end of the smaller tube and at the same time allows freedom of motion of the lower end. A short side neck provides for the attachment of a rubber tube and pipette bulb for forcing alcohol through the needle. As seen in plate 7, top, the needle and its supporting tube is moved by the system of levers in the same directions as the hand, but through only one-fourth or one-eighth the distances. A coil spring pulls the needle and its system of levers up and away from the dissection when it is released by the hand.

The frame supporting the parts just described has a spring clamp by which the whole piece of apparatus can be quickly attached to the casing of the vibrator or a piece of tubing and brought into position for use by means of the racks and pinions. In dissecting, the first and second fingers can be steadied on the hand rest shown in plate 6, top. With this instrument the needle is supported not by the hand but by a mechanism, and needs only to be guided by the hand. If released unexpectedly, it does not fall into the work but flies up and out of the way. By means of this mechanical device, nerves can be dissected and followed much more easily than when in sections, and it can be used in many situations in which the knife is not appropriate. It sometimes happens that a very fine (no. 12) needle can be used effectively when it is ground to a fine point and soldered to the knife clip in place of a knife.

Of the greatest use is a pair of fine-tipped forceps, which can be fashioned out of a pair of good, ordinary, fine forceps by soldering to the inner surfaces of the tips two no. 12 needles, accurately aligned parallel to each other with their points exactly opposite, and then, under a binocular microscope, grinding the needles to make them slender and with flat opposing surfaces.

Another valuable tool is a brush made from no. 52 white silk thread in the following way. A 6-inch piece is stretched between the ends of a U-shaped, stout wire. At intervals of $\frac{3}{4}$ -inch to 1 inch a minute drop of melted paraffin is applied, which cools instantly with sharply demarcated boundaries. Between the paraffin spots the rest of the thread is soaked with moderately thin glue (sinew glue) so that, on drying, the thread is embedded in a tough slender cylinder of glue not much greater in diameter than the thread itself. The thread may now be cut in the middle of each patch of paraffin and the middle of each section of glue, and the paraffin dissolved out with chloroform. Each piece then consists of a stiff cylinder with a tuft of loose fibers at one end, and

can be mounted or set with glue in a piece of glass tubing drawn down to capillary proportions. Brushes of several sizes can be made by cutting away as many strands as experience dictates. The finest need contain no more than 6 to 12 fibers.

Of considerable importance is the holding of the embryo. After use of more elaborate methods the following simple device has been found very satisfactory. To pieces of glass of uniform thickness and about $\frac{9}{16} \times \frac{11}{16}$ inches the embryos are attached with thick nitrocellulose, which should be so transparent that, later, in photographing, it will not interfere with the formation of a clear background. As shown in plate 6, top and bottom, the piece of glass is held by two screws with large, thin, flat heads to the flat side of a half-sphere of brass (diameter, $1\frac{1}{2}$ inches) having a central opening (as in pl. 7, bottom, c) and resting in a brass socket also having a large central aperture. Since the holder is really less than a hemisphere the embryo lies at about its center of curvature and is easily oriented without being moved out of the field even under high magnification. This holder can also be used in photographing.

The dissecting dish rests on a small, easily rotating turntable. Ordinarily it should be centered under the binocular, but sometimes it is useful to have it a little off center, and to use the horizontal circular motion to move tissue against the knife in cutting.

Plate 6, top, shows how the microscope, the stand for the dissecting instrument, and the resistance lamps are mounted on a baseboard. The board is wired, has two simple sockets for attaching the illuminator, and has two canopy switches, one for the illuminator, the other to control the dissecting instrument. The illuminator is set on an independent block to allow greater freedom in adjusting the light.

PHOTOGRAPHING EQUIPMENT

Stereoscopic photographs consist of a pair of photographs of the same object made from two different points of view corresponding to the vision of the two eyes. Such photographs may be made simultaneously with two lenses, as with a camera made to fit a binocular microscope, or in succession with one lens on an ordinary camera, which must then be moved from one position to another. The latter method was used in making the photographs illustrating this paper, and is involved in the construction of the camera. It has the advantage of utilizing the same lens for both pictures and of employing a type of lens (such as microsummars of 35 mm. and 64 mm.) which, because of its size if used in pairs, could not be brought to bear on the object.

In ordinary vision at reading distance the angle between the axes of the two eyes is 10 to 15 degrees. In the camera the angle between the two positions at which the exposures are made is 15 degrees at all magnifications. The simplest way to attain this end is to mount the bellows so that they can be inclined first to one side of the upright position and then to the other. Unless the embryo is in the exact axis of inclination the image will both move on the ground glass and be thrown out of focus when the camera is inclined.

All the main features, and many of the details, of the construction of the

camera may be observed in plate 8, top and bottom, such as: the composite bellows made in sections, the vertical post, mechanism for supporting and inclining the latter, the gears and vertical rod for operating the fine adjustment of the camera lens, the "stage" for supporting the embryo, with its background illuminator, and pulleys and belts (2 for horizontal control [by means of racks and pinions attached to a piece of glass forming the top of the stage] and one for vertical focusing of the embryo). The whole of the above-described mechanism is mounted on a heavy, cast iron base which in turn is supported on rubber cushions.

The holder for the embryo must allow both surface illumination and transmitted light for the background, and must permit any orientation of the embryo when the photographing is being done. Two types of holder have been employed to take the piece of glass with the embryo attached, and are shown in plate 7, bottom, *a* and *c*, the second being made from half of a bronze ball resting in a socket. Embryos cleared in benzyl-benzoate, which dissolves celloidin, must be held between two coverglasses which are in turn a part of the device shown in plate 7, bottom, *b*. The latter can be substituted for the central part of plate 7, bottom, *a*. Similarly, the little device at the left in plate 7, bottom, *c* is for holding a coverglass and lowering it onto an embryo placed on the glass covering the central opening.

The background illuminator (pl. 8, top and bottom) consists of an elbow of thin-wall brass tubing lined with plaster of Paris and fitted in the longer arm with a 6-c.p. automobile lamp. The intensity of the light is controlled by the position of the lamp in the tube and by a rheostat. It furnishes an even white background, and may be turned on or off independently of the lamps illuminating the embryo.

During an exposure, in order to keep the surface of the alcohol from being agitated and the image on the plate from being blurred, a coverglass held in a little clip on the end of the jointed arm of a simple rack-and-pinion stand (pl. 8, top) is carefully lowered over the embryo until it touches the alcohol and is nearly on a level with its surface. It is necessary to make the final focusing on the groundglass with the coverglass in place.

The surface illumination of the embryo is accomplished by a battery of 6 lamps (pl. 8, top) like those described for dissecting. The lamps are adjusted independently of one another and are attached in common to a movable base.

Although the post is graduated for different magnifications for each lens, it is necessary, in setting up the camera for any one lens and magnification, to adjust both the lens and the embryo. The former is done by (1) slipping into the opening in the center of the horizontal steel axis pin of the inclination joint, a straight rod, the free, tapered end of which reaches to the optical axis of the camera, and (2) bringing the image of the tapered tip into sharp focus in the center of the groundglass. The rod is then removed, and the embryo placed on the stage and brought into the desired focus by raising and lowering it. This focusing of the embryo as well as centering it on the groundglass may be accomplished at any height by means of the three vertical rods which operate the pulleys and belts.

SUMMARY

A new method has been developed for dissecting embryos by means of electrically operated knives made from safety-razor blades, and needles (made from hypodermic needles) held and delicately controlled by a system of levers. Other aids are very fine-tipped forceps, and brushes made from a few strands of fine silk thread. A new illuminator consists of a combination of spherical and elliptical reflectors by which light from a small flashlight bulb is concentrated, with scarcely perceptible heat, on a very small area. The old principle of stereoscopic photography has been applied to the illustrating of embryology. For this purpose a camera has been adapted to the photography of whole embryos and of various dissections. For both dissecting and photographing, holders for the embryo which use the principle of the ball-and-socket joint, have been devised.

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EXPLANATION OF PLATES

PLATE 6

Top. $\times \frac{1}{4}$. General view of the microscope, turntable and dissecting dish, and the dissecting instrument (on its stand) connected by coiled lead wires to the lamps, all mounted on a baseboard; and just beyond, the illuminator and its transformer on a separate base and connected by lead wires to the sockets in the baseboard. The latter has binding posts (at the right) and two canopy switches, one for the illuminator and the other for the dissecting instrument (at the corners on either side of the post of the microscope). There is an adjustable hand rest between the observer and the turntable and dissecting dish.

Bottom. \times about $\frac{3}{4}$. Enlarged view of the dissecting instrument, embryo holder, dish and turntable. At *a* are shown the magnets, armature, and balancing springs of the vibrating mechanism with the glass cover removed. At *b* are three knives of different sizes, on their clips. The dissecting dish is about 2 inches high, and $2\frac{3}{4}$ inches in diameter.

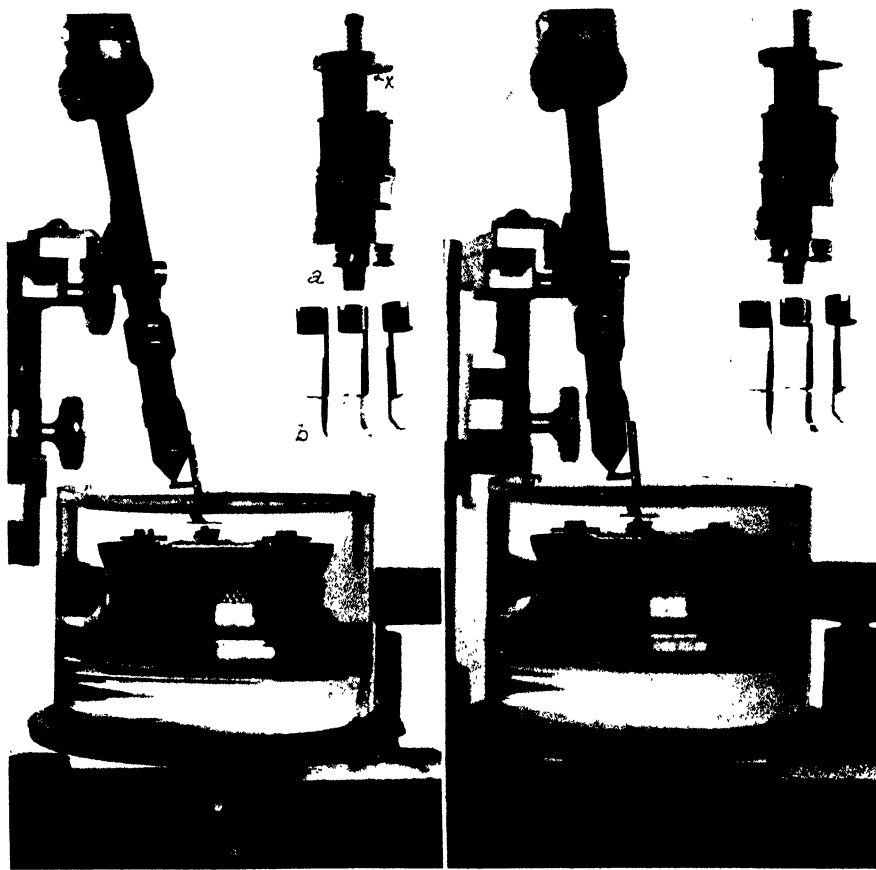
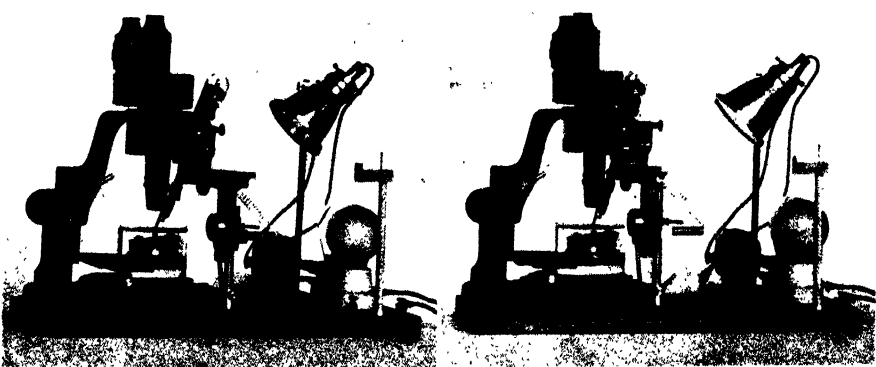


PLATE 7

Top. $\times \frac{1}{2}$. Dissecting-needle holder and manipulator, showing how it may be attached by a spring clamp to the casing of the dissecting instrument by which it is supported. A hypodermic needle, ground down to a fine point, is used because alcohol (in which the dissecting is conducted) can be forced through it to wash away the loosened material, and allowed to run back again. At *a* is shown a pneumatic forceps which can be slipped on the needle holder in place of the needle. The system is filled with air instead of alcohol. A rubber tubing can be attached to one of the arms, which is made from a hypodermic needle, and used for forcing alcohol through the needle to wash away debris.

Bottom. $\times \frac{1}{2}$. Devices for holding embryos while they are being photographed. In *a* and *c* the embryos are attached to small pieces of glass (like the smaller in *c*) which are held by the screws in *a*, and by the clips in *c*. Embryos that cannot be attached to glass by celloidin, as, for example, embryos injected with ink and cleared in benzyl-benzoate, are gently pinched between two coverslips by the device at *b*, which is used in the frame of *a*. The coverslips are practically invisible in the photograph.

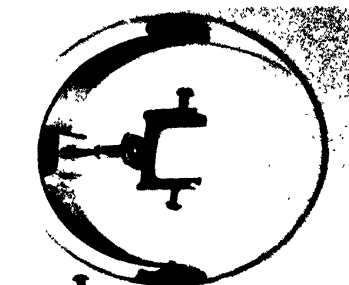
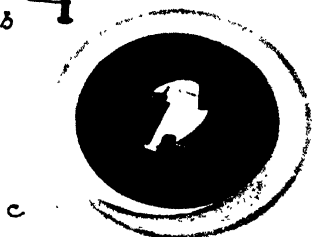
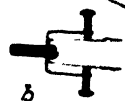
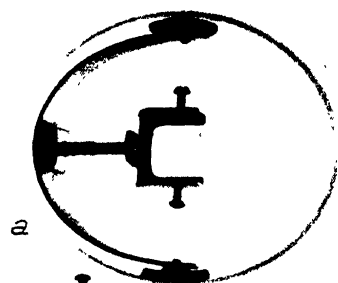
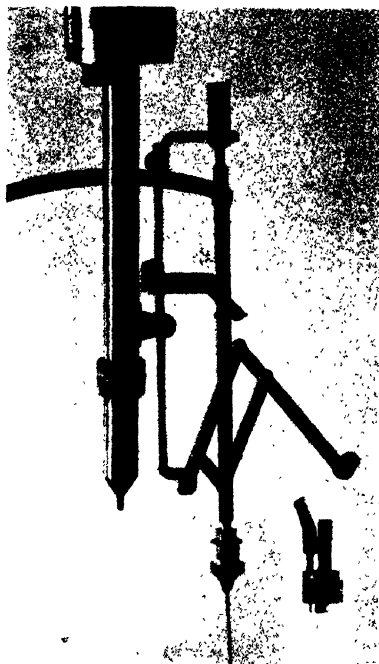
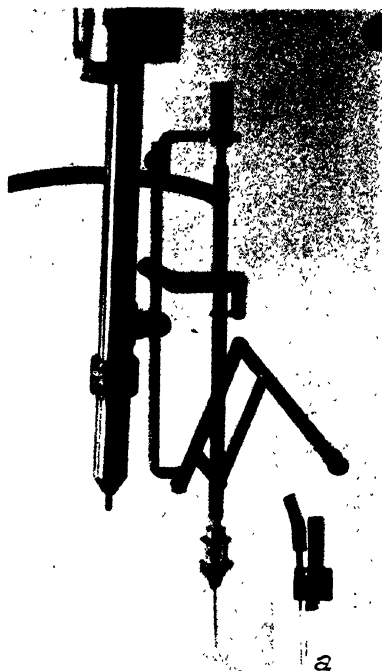
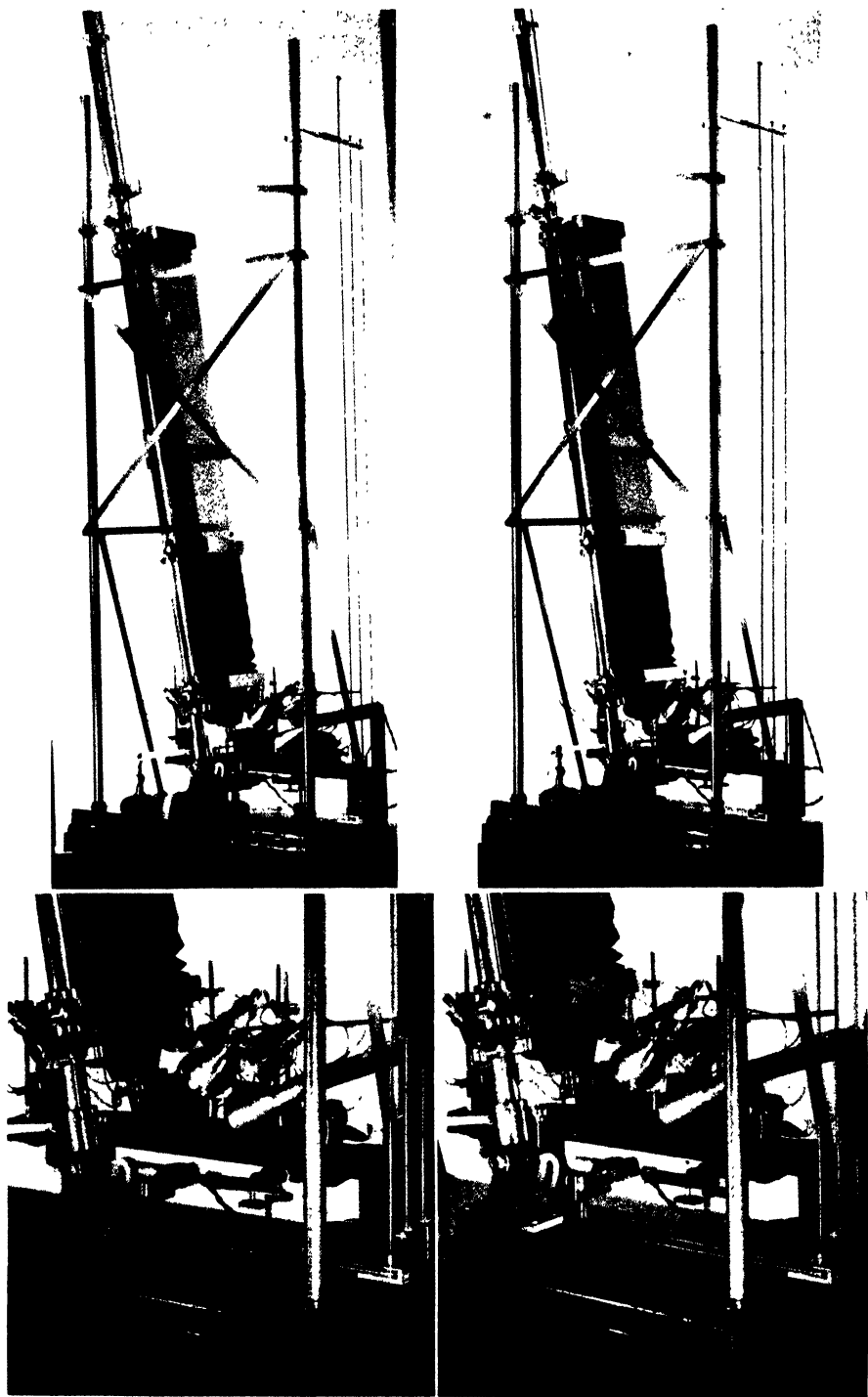


PLATE 8

Top. \times about $\frac{1}{18}$. General view of the camera and lamps. Shows the base, and frame for supporting the bellows in an inclined position. The three slender vertical rods and their attached pulleys and belts enable the user, even when working at the top of a six-foot bellows, to bring the embryo into focus and its image into the center of the groundglass.

Bottom. \times about $\frac{1}{6}$. Lower part of the camera, showing the gears of the fine adjustment for focusing the lens, part of the mechanism for inclining the vertical post (with the camera bellows) on the horizontal axis, the two horizontal movements by rack and pinion of the stage, the vertical fine adjustment for bringing the embryo into focus in the axis of inclination, and the background illuminator (which is lighted) carried on the stage.



**A REVIEW OF THE PHYLLODOCIDAE
(ANNELIDA POLYCHAETA) OF THE
COAST OF CALIFORNIA, WITH
DESCRIPTIONS OF NINE NEW SPECIES**

**BY
OLGA HARTMAN**

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A REVIEW OF THE PHYLLODOCIDAE (~~ANNELIDA~~ POLYCHAETA) OF THE COAST OF CALIFORNIA, WITH DESCRIPTIONS OF NINE NEW SPECIES

BY

OLGA HARTMAN

THE NINE new species of Phyllodocidae described in the following pages, together with those not heretofore reported from California, bring the total for California to twenty-nine species in thirteen genera. The following revised list includes fifteen species previously known from California, with new synonyms as indicated, five species previously described but heretofore not reported from California, and nine species new to science. Species new for California are preceded by an asterisk. Holotypes are deposited in the United States National Museum, paratypes in the University of California.

LIST OF SPECIES OF PHYLLODOCIDAE FROM CALIFORNIA

1. *Anaitides heterocirrus* Chamberlin, 1918
2. *Anaitides medipapillata* (Moore), 1909
3. *Anaitides mucosa* (Ersted), 1843
- *4. *Anaitides longipes* (Kinberg), 1866
- *5. *Anaitides williamsi* sp. nov., p. 125
- *6. *Clavadoce splendida* gen. et sp. nov., p. 123
7. *Eteone pacifica* Hartman, 1936
- Eteone maculata* Treadwell, 1922, *non* Ersted, 1843
- *8. *Eteone dilatata* sp. nov., p. 130
- *9. *Eteone lighti* sp. nov., p. 127
- *10. *Eteone californica* sp. nov., p. 131
- *11. *Eteone balboensis* sp. nov., p. 131
12. *Eulalia levicornuta* Moore, 1906
- *13. *Eulalia quadrioculata* Moore, 1906
- *14. *Eulalia strigata* Ehlers, 1901
- *15. *Eulalia avisculiseta* sp. nov., p. 122
16. *Eumida longicornuta* (Moore), 1906
17. *Eumida tubiformis* Moore, 1909
- *18. *Eumida sanguinea* (Ersted), 1843
19. *Genetyllis castanea* (Marenzeller), 1879
20. *Genetyllis nigrimaculata* (Moore), 1909
- Eulalia nigrimaculata* Moore; Berkeley, 1924
- This species is characterized by having segments 1 and 2 coalesced and more or less reduced as in the genus *Genetyllis*.
21. *Hesperophyllum tectum* Chamberlin, 1919
- *22. *Hypoeulalia* sp.
- This I know only from a single individual having a bifurcated median prostomial antenna. Its description awaits additional material.
23. *Notophyllum imbricatum* Moore, 1906
24. *Paranaitis polynoides* (Moore), 1909

25. *Phyllodoce ferruginea* Moore, 1909

26. *Sige bifoliata* (Moore), 1909

† *Sige californiensis* Chamberlin, 1919

Berkeley, 1924, has already shown that Moore's species is a true *Sige*, and has corrected the original descriptions of the peristomial cirri and median antenna. So far as the description of *S. californiensis* goes, it presents no differences from Moore's species.

*27. *Sige montereyensis* sp. nov., p. 126

28. *Steggoa gracilior* Chamberlin, 1919

*29. *Steggoa californiensis* sp. nov., p. 122

KEY TO SPECIES OF PHYLLODOCIDAE FROM CALIFORNIA

1. With 4 pairs of tentacular cirri (fig. 1) 2
1. With 2 pairs of tentacular cirri (fig. 47) .. *Eteone* 22
2. Parapodia biramous 3
2. Parapodia uniramous (fig. 4) 4
3. Ventral cirrus of segment 2 similar to other tentacular cirri. *Notophyllum imbricatum*
3. Ventral cirrus of segment 2 asymmetrical and foliaceous. *Hesperophyllum tectum*
4. First segment fused with prostomium, second and third segments free. *Hypoeulalia* sp.
4. Segments 1 and 2 more or less fused but free from prostomium. 20
4. Segments 1 and 2 free but sometimes more or less reduced dorsally. 5
5. Tentacular segments complete rings (fig. 1); prostomium with median antenna; proboscis diffusely papillated (fig. 2) 6
5. First segment dorsally reduced; prostomium with or without median antenna. 10
5. First segment dorsally and ventrally reduced, second segment dorsally reduced (fig. 14); prostomium with median antenna (fig. 14) *Clavadoce splendida*, p. 123
6. Tentacular cirri cirriform (fig. 1) .. *Eulalia* 7
6. Ventral cirrus of segment 2 foliaceous, asymmetrical; other tentacular cirri cirriform. *Steggoa* 9
7. Dorsal cirri of median parapodia elongate, lanceolate (fig. 3) 8
7. Dorsal cirri of median parapodia subrectangular, truncate distally (fig. 11)
Eulalia strigata
7. Dorsal cirri of median parapodia subovate or subcordate. *Eulalia levicornuta*
8. Prostomium with 2 lateral eyespots besides a pair of lenticulated eyes; dorsal cirri inflated, slender, elongate (figs. 7, 8, 9) *Eulalia quadrioculata*
8. Prostomium without lateral eyespots; dorsal cirri foliaceous (figs. 3, 4, 5)
Eulalia aviculiseta, p. 122
9. Without eyes; median antenna nearly as long as lateral antennae; notocirri lanceolate *Steggoa gracilior*
9. With eyes; median antenna minute (fig. 18); notocirri truncate distally (figs. 19, 21, 22) *Steggoa californiensis*, p. 122
10. Prostomium with median antenna. 11
10. Prostomium without median antenna, with or without nuchal papilla. 15
11. Tentacular cirri all filiform. *Eumida* 12
11. Ventral tentacular cirrus of segment 2 foliaceous, others filiform; proboscis diffusely papillated. *Sige* 14
12. Posterior neuropodia slender, greatly elongated, more than two and one-half times as long as deep, distally truncate (fig. 15); dorsal and ventral cirri distally somewhat pointed (fig. 15) *Eumida tubiformis*
12. Posterior neuropodia not greatly elongated. 13
13. Posterior dorsal cirri deeper than long, with a very wide base (fig. 17), wider than half of width of dorsal cirri; cirri strongly imbricated; prostomium broader than long *Eumida longicornuta*

13. Posterior dorsal cirri longer than deep (fig. 16), held more or less erect; base of attachment of dorsal cirri less than half as wide as cirrus; prostomium as long as wide or longer *Eumida sanguinea*
14. Dorsal cirri cordate (figs. 23, 25), less than twice as long as deep; prostomium with a straight posterior margin..... *Sige bifoliata*
14. Dorsal cirri lanceolate, more than twice as long as deep (figs. 27, 28); prostomium incised at posterior median margin..... *Sige montereyensis*, p. 126
15. Proboscis proximally set with longitudinal rows of papillae.. *Anaitides*..... 16
15. Proboscis proximally set with diffuse papillae..... *Phyllodoce ferruginea*
16. First 3 normal segments with parapodia and cirri greatly reduced. *Anaitides heterocirrus*
16. First 3 normal segments with parapodia not reduced..... 17
17. Notocirrophores greatly elongated, stalklike; notocirri reniform.... *Anaitides longipes*
17. Notocirrophores domelike or flattened; notocirri not reniform..... 18
18. Prostomium broadest in anterior half, hexagonal in outline; median notocirri distally pointed; larger..... *Anaitides medipapillata*
18. Prostomium broadest in posterior half, cordate; smaller..... 19
19. Prostomium longer than broad (fig. 35); neurocirri bluntly rounded distally (figs. 33, 34); with conspicuous pigmentation consisting of three longitudinal lines on dorsal and ventral sides..... *Anaitides williamsi*, p. 126
19. Prostomium as long as broad; neurocirri pointed distally; without conspicuous pigmentation pattern..... *Anaitides mucosa*
20. Segments 1 and 2 fused and well developed; nuchal papilla present. *Paranaitis polynoides*
20. Segments 1 and 2 fused, dorsally reduced, ventrally well developed; nuchal papilla absent.. *Genetyllis* 21
21. Dorsal cirri cordate; prostomium with median antenna; color in life blue with metallic iridescence..... *Genetyllis nigrimaculata*
21. Dorsal cirri ovate to ovate-cuneate; prostomium without median antenna; color in life salmon to reddish brown..... *Genetyllis castanea*
22. Larger, more than 50 mm. long; dorsal cirri broadly rounded (figs. 47, 48), subcordate; ornamented with irregularly spaced black spots..... *Eteone pacifica*
22. Smaller, less than 50 mm. long, dorsal cirri not cordate; not ornamented with black spots 23
23. Prostomium broader than long (fig. 36); first segment dorsally reduced (fig. 36); dorsal cirri distally pointed (figs. 37, 39), approximately triangular in shape
Eteone lighti, p. 127
23. Prostomium as long as broad or longer; first segment fully developed dorsally (fig. 42) 24
24. Parapodia anteriorly tiny, appearing dwarfed because of inflated nature of anterior body segments; prostomium long, slender, trapezoidal (fig. 42).. *Eteone dilatata*, p. 130
24. Parapodia well developed, provided with thickened cirri and stout glandular parapodial bases; prostomium as long as broad or slightly longer, tending to be conical.. 25
25. Prostomium with eyes (fig. 43); dorsal cirri as broad as long throughout length of body (figs. 44, 45)..... *Eteone californica*, p. 131
25. Prostomium without eyes (fig. 49); median dorsal cirri rectangular in outline and longer than broad (fig. 50)..... *Eteone balboensis*, p. 131

Figs. 1-6. *Eulalia aviculiset*

- Fig. 1. Anterior end including first 3 segments, in dorsal view ($\times 20$).
Fig. 2. Prostomium and protruded proboscis, from left side ($\times 8$).
Fig. 3. A posterior dorsal cirrus ($\times 74$).
Fig. 4. A median parapodium, in posterior view ($\times 74$).
Fig. 5. An anterior parapodium, in posterior view ($\times 74$).
Fig. 6. Seta from a median parapodium ($\times 693$).

Figs. 7-10. *Eulalia quadrioculata*

- Fig. 7. Anterior parapodium in posterior view ($\times 50$).
Fig. 8. Posterior parapodium in posterior view ($\times 50$).
Fig. 9. Median parapodium in anterior view ($\times 50$).
Fig. 10. Seta from a median parapodium ($\times 693$).

Figs. 11, 12. *Eulalia strigata*

- Fig. 11. A median parapodium ($\times 135$).
Fig. 12. A posterior parapodium ($\times 135$).

Figs. 13, 14. *Clavadoce splendida*

- Fig. 13. Twenty-third parapodium in anterior view; dorsal cirrus detached. Setae diagrammatically shown ($\times 20$).
Fig. 14. Anterior end, including prostomium and first 4 segments ($\times 42$).

- Fig. 15. *Eumida tubiformis*. A parapodium from posterior third of body ($\times 61$).
Fig. 16. *Eumida sanguinea*. A parapodium from posterior third of body ($\times 61$).
Fig. 17. *Eumida longicornuta*. A parapodium from posterior third of body ($\times 61$).



Figs. 1-17

DESCRIPTION OF NEW SPECIES

Eulalia aviculiseta sp. nov.

(figs. 1-6)

Diagnosis.—Length 18 to 25 mm.; width 1.5 to 2.0 mm.; number of segments 70 to 85. Prostomium longer than broad, dorsally convex, with a rounded anterior border and a subrectangular posterior margin, having at its anterior margin 4 tapering antennae about two-thirds as long as the prostomium, and a median antenna which is slightly slenderer and longer and inserted somewhat posterior to the middle of the prostomium (fig. 1). Eyes, one pair, crescentic or transversely oval in shape, near posterior dorsolateral part of prostomium (fig. 1).

Tentacular cirri symmetrically cirriform except for ventral two, which are thicker near base and somewhat asymmetrical, the others resembling the dorsal cirri but tapering rapidly to a point; tentacular cirrophores well developed (fig. 1).

Segments well differentiated throughout length, separated by deeply pigmented, constricted interpodal rings (fig. 1) which are narrowest in anterior third of body and come to be half as wide dorsally as segments are long in posterior half of body; interpodal ring unpigmented ventrally.

Parapodia somewhat ventrolateral in anterior two-thirds, lateral in posterior region; neuropodium extending laterally beyond dorsal cirrophore (fig. 4), with cleft preacicular lobe exceeding in length the rounded postacicular lobe, and with thickened, slightly compressed, subconical, ventral cirrus (fig. 4). Dorsal cirri pointed, asymmetrical, in first 10 to 15 segments, becoming lanceolate in more posterior segments, attached by a broad base (figs. 3, 4) two and one-half to three and one-half times as long as broad.

Proboscis diffusely papillated except for a smooth, narrow oral ring which may cover one-third of the proximal surface; distal end provided with 20 crenulate papillae (fig. 2).

Setae pale, composite; shaft with slightly thickened articulating end, with numerous fine hairs and 2 beaklike teeth (fig. 6); appendage less than 7 times as long as wide at base and distinctly toothed on its external border.

Color drab olive or taupe dorsally, with dark to black intersegmental grooves dorsally; prostomium pale but with dark brown eyes.

Distribution.—Moss Beach, San Mateo County (type); Pacific Grove, Monterey County, north to Fort Bragg, Mendocino County, California.

Systematic discussion.—*Eulalia aviculiseta* approaches *E. viridis* (O. F. Müller) in its body proportions, in having lanceolate cirri, and in the general shape of its parapodial lobes. It differs from *E. viridis* in at least the following details: (1) the prostomium is longer than wide and has transversely oval eyes which are set close to the posterior margin of the prostomium; (2) the median antenna is inserted near the middle of the prostomium; (3) the setal shaft has beaklike distal teeth; and (4) the dorsum is strikingly marked by dark interpodal bands.

Steggoa californiensis sp. nov.

(figs. 18-22)

Diagnosis.—Long, slender, length to 50 mm.; width 2 to 3 mm.; number of segments 400 or more. Prostomium subquadrate except for narrower anterior part at the sides of which frontal antennae are attached, and having near its posterolateral border a pair of relatively small black eyes separated by a distance 3 times their width; paired prostomial antennae tapering, about two-thirds as long as prostomium, directed laterally; median antenna tiny, inserted in line with anterior border of eyes (fig. 18).

Tentacular cirri inserted on low cirrophores, their styles relatively short, the longest, on segment 3, about two and one-half times as long as the first normal dorsal cirrus; second ventral cirrus asymmetrically flattened but with acute tip, about three-fourths as long as dorsal cirrus 2, but wider. Neuropodium of segment 2 without setae.

Parapodia ventrolateral; neuropodium with rounded setal lobe, presetal lobe slightly longer (fig. 21); aciculum projecting slightly beyond lobes; cirrophores rudimentary. Dorsal cirri pale, thickened, some with suggestion of acuminate tips, most with broad, truncate distal margins and abrupt bases (figs. 19, 21, 22), marked by coarsely pinnate glandular areas. Setae pale, 9 to 14 in a fascicle, linear; distal end of shaft somewhat enlarged and covered with fine hairs on one side; appendage with minute, widely separated denticulations (fig. 20).

Proboscis clavate when protruded, closely covered with about 8 longitudinal rows of transversely elongate, low mounds largest at distal end, gradually becoming tiny at oral end.

Anal cirri dorsolateral, cylindrical, about as long as last 3 segments; anal ring with tiny median pointed papilla on dorsal side.

Color pale flesh, dorsal cirri greenish brown, dorsal side of all segments with a median dark green patch and a pair of dorsolateral transverse dashes, resulting in a dorsal pattern resembling 3 broken, longitudinal lines; a tiny dark spot just within the ventral border of each parapodium.

Distribution.—Tomales Bay, Marin County (type); Fort Bragg, Mendocino County, south to Point Conception, Santa Barbara County, California.

Systematic discussion.—Only 3 previously described species have been attributed to *Steggoa* Bergström, all from the Pacific Ocean. *S. brevicornis* (Ehlers) from New Zealand has cordate notocirrophores (*vide* Augener, 1913, p. 20), *S. magalaensis* (Kinberg, 1865) from the Straits of Magellan has greatly elongated dorsal cirri, and *S. gracilior* Chamberlin from Laguna, California, is said to lack eyes. Chamberlin's type is no more extant and the type locality has been seriously invaded and partly destroyed. From all of these species, *S. californiensis* differs in the shape of its notocirri, in the proportions of its prostomium, and in having eyes.

CLAVADOCE gen. nov.

Type species.—*C. splendida* sp. nov.

Diagnosis of genus.—Prostomium with 2 pairs of anterolateral clavate antennae; first 3 segments free from one another and from prostomium, but segment 1 greatly reduced and present only as lateral expansion from which first pair of tentacles arise; segment 2 reduced dorsally but well developed ventrally; segment 3 normally developed. Formula of tentacular cirri: $1 + S\frac{1}{I} + S\frac{1}{N}$. Parapodia uniramous, with only composite setae. Dorsal cirri foliaceous.

Differs from *Eumida* Malmgren and *Pirakia* Bergström in having segment 1 dorsally and ventrally reduced and segment 2 dorsally reduced; also in that tentacular cirri are clavate instead of filiform.

Type locality.—Tomales Bay, Marin County, California.

Clavadoce splendida sp. nov.

(figs. 13 and 14)

Diagnosis.—Almost uniformly broad, depressed for about three-fourths of its length, tapering anteriorly and posteriorly to blunt ends. Length 20 mm.; width without parapodia, 1.0 mm., with, 2.5 mm.; number of segments 77.

Prostomium broader than long, truncate (fig. 14). Eyes 2, large, almost one-fourth as wide as prostomium. Median antenna inserted near anterior margin (fig. 14); paired an-

Figs. 18-22. *Steggoa californiensis*

- Fig. 18. Anterior end in dorsal view ($\times 42$).
Fig. 19. An anterior parapodium ($\times 74$).
Fig. 20. Seta from a median parapodium ($\times 693$).
Fig. 21. A median parapodium in anterior view ($\times 74$).
Fig. 22. A posterior parapodium ($\times 74$).

Figs. 23-26. *Sige bifoliata*

- Fig. 23. Dorsal cirrus from tenth parapodium ($\times 61$).
Fig. 24. Tenth neuropodium showing lobes and projecting aciculum ($\times 74$).
Fig. 25. Fortieth parapodium in anterior view ($\times 61$).
Fig. 26. Articulation of seta from fortieth parapodium ($\times 323$).

Figs. 27-29. *Sige montereyensis*

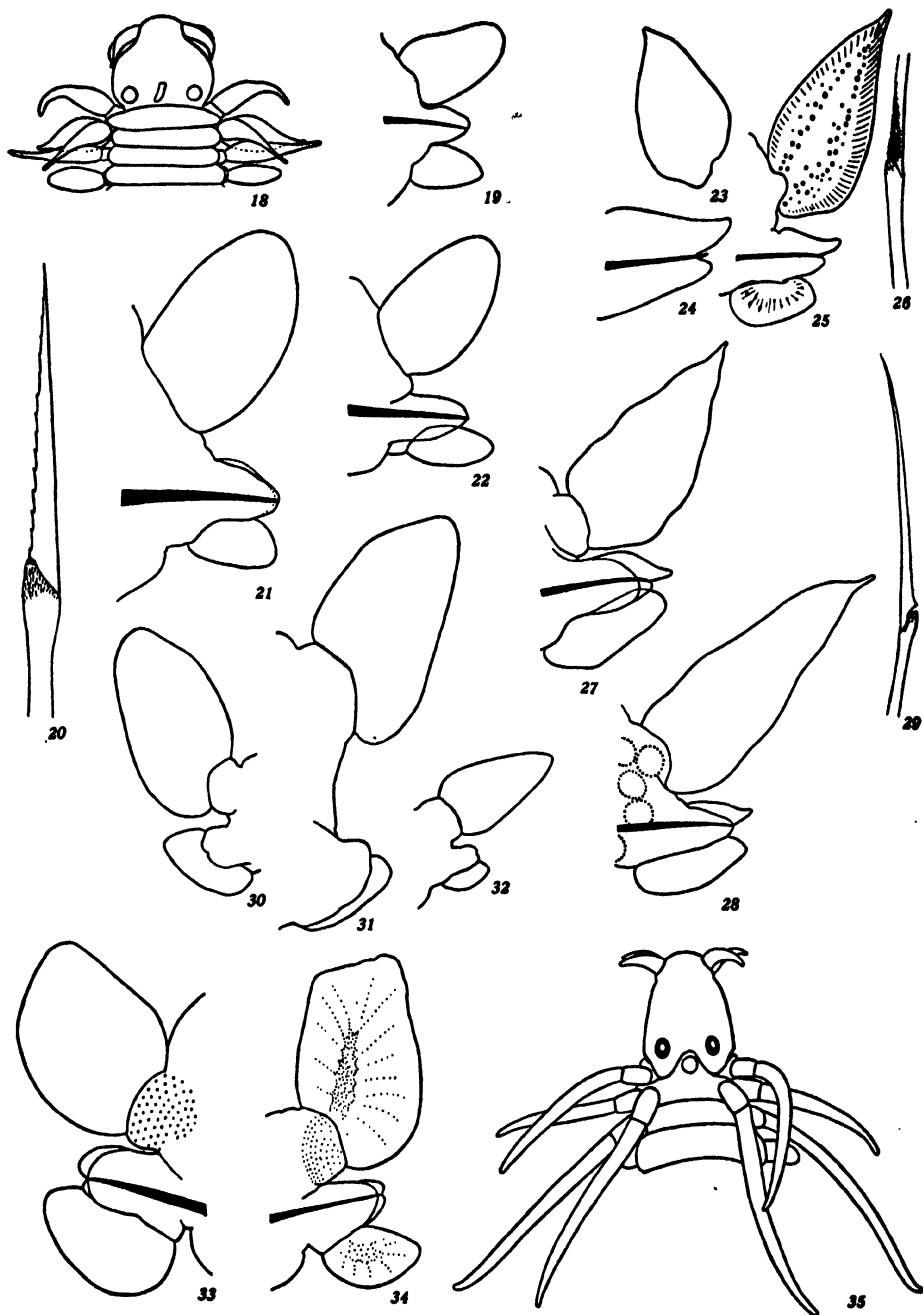
- Fig. 27. Twentieth parapodium ($\times 74$).
Fig. 28. A posterior parapodium, with eggs ($\times 74$).
Fig. 29. Seta from a posterior parapodium ($\times 323$).

Figs. 30-32. *Phyllodoce ferruginea*

- Fig. 30. An anterior parapodium ($\times 42$).
Fig. 31. A median parapodium ($\times 42$).
Fig. 32. A posterior parapodium ($\times 42$).

Figs. 33-35. *Anaitides williamsi*

- Fig. 33. Anterior parapodium in posterior view ($\times 150$).
Fig. 34. Posterior parapodium from another individual, in posterior view ($\times 60$).
Fig. 35. Anterior end in dorsal view ($\times 42$).



Figs. 18-35.

tennae similar in shape but differing in size, the second dorsal being the largest, the second ventral the smallest (fig. 14).

Parapodia lateral, provided dorsally with globular cirrophores (fig. 13) and foliaceous cirrostyles which are broadly imbricated and cover dorsum at least in anterior half of body, smaller posteriorly. Dorsal cirri strongly asymmetrical; ventral cirri thick, inflated, those from successive segments touching, auricular in outline (fig. 13), about twice as deep as long. Setae composite, in fascicles of about 20.

Locality.—Known from a single individual collected from *Zostera-Ulva* flats of Tomales Bay, Marin County, California.

Sige montereyensis sp. nov.

(figs. 27–29)

Diagnosis.—Length 8 mm.; width about 1.0 mm.; number of segments 67; relatively short, depressed, dorsum and ventrum moderately convex; median and posterior segments faintly triannulated.

Prostomium pale, bluntly rounded anteriorly, deeply incised in posterior median line. Prostomial antennae inserted at anteroectal border, their length slightly exceeding that of prostomium. Median antenna not observed. Eyes dark, conspicuous, lenticular, separated by a distance equal to their diameter.

Segment 1 dorsally reduced, represented in middorsal area by a triangular portion contained within prostomial notch; second dorsal cirrus longest, about as long as first 10 segments, ventral of segment 2 shortest, provided with a broad, triangular, foliaceous lobe continued along ventral margin except for short, free, distal tip, the lobe almost twice as deep as that of typical ventral cirrus.

Parapodia uniramous; neuropodia produced into elongate conical supra-acicular lobes (figs. 27, 28) surpassing the ventral cirrus in length, and a shorter subacicular lobe. Notocirrophores low, dome-shaped, with thin, strongly foliaceous asymmetrical dorsal cirri throughout; dorsal cirri overlapping, longer than broad and becoming gradually more so in posterior region (fig. 28), so that in last few segments length is about equal to 4 times width; ventral cirri thin, tapering slightly distally, projecting laterally beyond inferior acicular lobe. Setae colorless, slender, numbering about 20 in anterior parapodia and gradually decreasing to 16 or fewer in posterior parapodia; articulation slightly thickened, ornamented with a few inconspicuous spines but without teeth (fig. 29).

Locality.—Monterey, California, in 6–8 fathoms. A single individual collected by Mr. E. F. Ricketts, June, 1934.

Systematic discussion.—*Sige montereyensis* approaches *Sige macroceros* (Grube) in its prostomial proportions, but differs from the latter in the shape of its prostomium, which is subcordate, and in having relatively longer dorsal cirri in posterior half of body.

Anaitides williamsi sp. nov.

(figs. 33–35)

Diagnosis.—Long, uniformly slender; length to 40 mm.; width without notocirri to 2 mm.; segments about 200. Prostomium convex, depressed, longer than wide, narrowest anteriorly, widest in region of eyes, with posterior median invagination in which the nuchal papilla is inserted (fig. 35). Eyes 2, reddish brown, situated at dorsolateral margin on posterior fourth of prostomium, relatively far apart, separated by a distance equal to 3 or 4 times their diameter. Prostomial antennae inserted at anterolateral margin of prostomium, short, as long as anterior width of prostomium (fig. 35). Lower lip thick, smooth, with a median incision.

Tentacular cirri cirriform, elongate (fig. 35), the shortest about as long as first 5 segments, the longest or dorsal 3 as long as first 8 segments.

Parapodia well developed throughout; neuropodium with an incised preacicular lobe exceeded in length by the neurocirri (figs. 33, 34). Setae in fan-shaped fascicles of 7 to 10. Notocirrophores low anteriorly, increasing in height in median and posterior segments. Notocirri foliaceous, asymmetrical, broadly oval in anterior and posterior segments (fig. 33), more or less quadrangular in median segments, with blunt, truncate distal margin (fig. 34) and glandular striations pinnately arranged. Neurocirri thick, fleshy, narrowly rounded distally, exceeding the neuropodia in length.

Proboscis proximally with 6 rows of papillae on each side, with 9 papillae in each series. Anal cirri 2, thickened proximally, gradually tapering distally, twice as long as width of anal segment.

Color pale; prostomium with small dark specks, dorsum and ventrum each conspicuously marked by 3 longitudinal dark bands, a median and 2 lateral, segmentally broken, the lateral bands extending over the parapodia and notocirrophores and a patch of the same dark pigment diffused near the middle of each notocirrus and faintly on each neurocirrus.

Named for Professor O. L. Williams, of the College of the Pacific, who contributed valuable collections, some of which have been used in these studies.

Distribution.—Tomales Bay (type) and Drake's Estero, both of Marin County, and San Francisco Bay northeast of the Golden Gate, California.

Systematic discussion.—*Anatides williamsi* approaches *A. mucosa* (Ertsted) as redescribed by Malmgren (1865) and Bergström (1914) in the number and arrangement of papillae on the proboscis, in the outlines of the dorsal cirri, and in general body proportions. It differs from *A. mucosa* in at least the following respects: (1) neurocirri are not pointed distally, but are thick throughout and blunt; (2) the prostomium is longer than wide; (3) the number of segments ranges to as high as 200; (4) notocirri are relatively longer in the median and posterior regions and have a slenderer cirrophore; and (5) the pigmentation pattern is different.

Eteone lighti sp. nov.

(figs. 36–39)

Diagnosis.—Length to 30 mm.; width with parapodia 1.0 to 1.5 mm.; number of segments 75 to 100. Prostomium trapezoidal, somewhat wider than long, width of anterior margin less than one-third that of posterior margin (fig. 36), with a median groove extending throughout its length. Prostomial antennae subulate, inserted at anteroecetal margin, their length less than that of anterior border of prostomium. Eyes 2, black, lozenge-shaped, situated on posterior third of prostomium (fig. 36).

Segment 1 dorsally reduced, produced laterally as an expansion (fig. 36) and ventrally forming a short lower lip; provided with a pair of cirriform tentacles but without setae or parapodial prominence.

Parapodia present from segment 2 to posterior end, the first pair about half as large as those following and without dorsal cirri. Parapodia prominent on other segments, their length exceeding half of body width posterior to segment 10, gradually becoming relatively longer so that in posterior fifth of body, segments are as long as wide. Neuropodial lobes gradually increasing in length posteriorly, projecting laterally beyond ventral cirri throughout (figs. 37, 39); pre- and postsetal lobes rounded, almost equal in length in anterior region, the postsetal lobe becoming gradually longer in posterior region but never greatly exceeding the presetal lobe.

Dorsal cirri conspicuous, more or less triangular in outline (fig. 37), tapering distally to blunt tips, proportions more or less constant throughout except for elongation posteriorly, length and width equal in anterior region, becoming gradually longer than broad in posterior half; cirrophores low, flat, broad. Ventral cirri broadly attached, tapering distally to blunt

Figs. 36-39. *Eteone lighti*

- Fig. 36. Anterior end in dorsal view ($\times 42$).
Fig. 37. Thirty-fifth parapodium in posterior view ($\times 152$).
Fig. 38. Articulation of seta from a posterior parapodium ($\times 693$).
Fig. 39. A posterior parapodium in posterior view ($\times 152$).

Figs. 40-42. *Eteone dilatae*

- Fig. 40. A median parapodium ($\times 152$).
Fig. 41. A posterior parapodium ($\times 152$).
Fig. 42. Anterior end in dorsal view ($\times 42$).

Figs. 43-46 *Eteone californica*

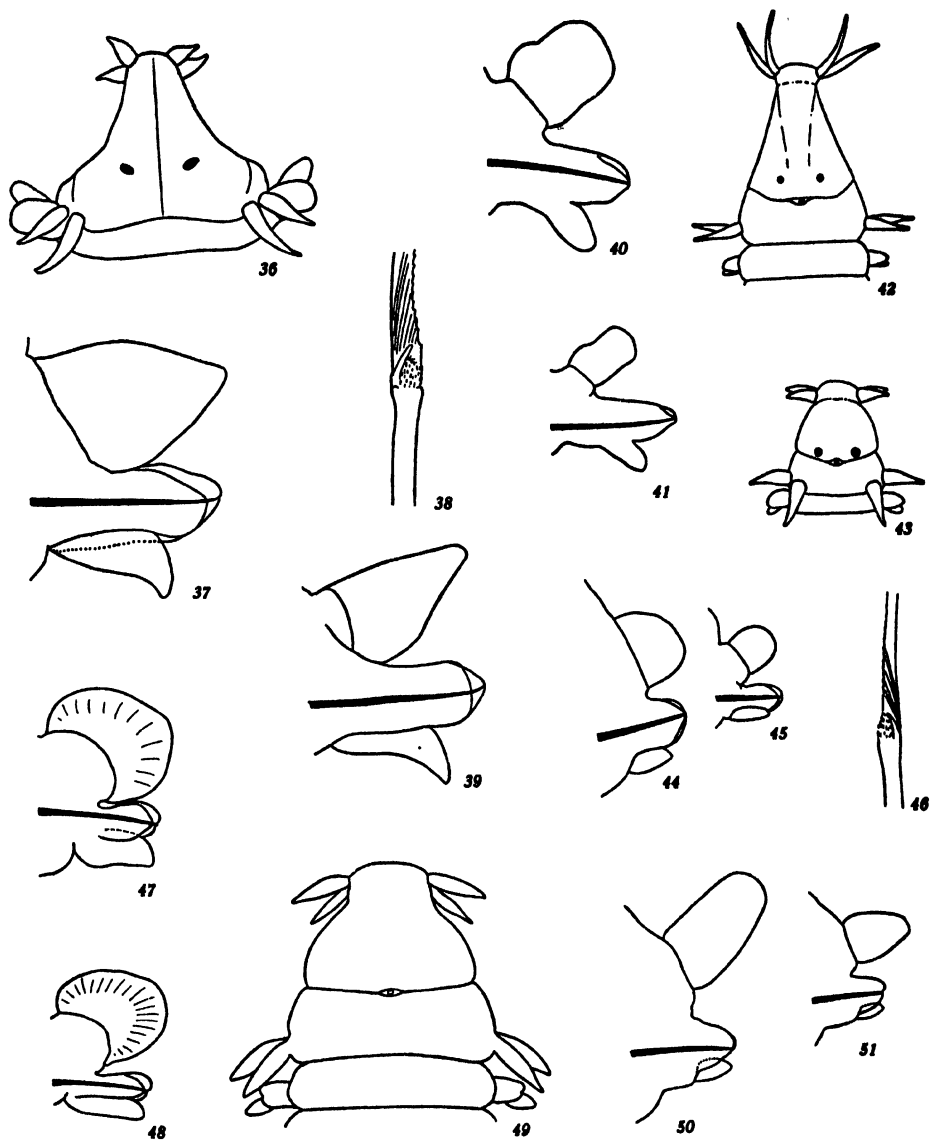
- Fig. 43. Anterior end in dorsal view ($\times 42$).
Fig. 44. A median parapodium ($\times 61$).
Fig. 45. A posterior parapodium ($\times 61$).
Fig. 46. Articulation of seta from a median parapodium ($\times 323$).

Figs. 47, 48. *Eteone pacifica*

- Fig. 47. A median parapodium in posterior view ($\times 20$).
Fig. 48. A posterior parapodium in posterior view ($\times 20$).

Figs. 49-51. *Eteone balboensis*

- Fig. 49. Anterior end in dorsal view ($\times 42$).
Fig. 50. A median parapodium in anterior view ($\times 61$).
Fig. 51. A posterior parapodium in anterior view ($\times 61$).



Figs. 36-51.

tip and directed somewhat ventrally (figs. 37, 39). Setae colorless, slender, pointed distally, 12 to 16 in a fascicle; articulation with a pair of lateral humps subterminally, a tooth and fine spines distally (fig. 38); appendage finely denticulated on concave edge.

Proboscis (dissected) smooth or slightly wrinkled but without papillae. Anal cirri 2, lateral, cirriform, with proportions similar to those of peristomial cirri but slightly more than twice as large.

Color pale or whitish with pale, fulvous transverse stripes dorsally; dorsal cirri tipped with similar but deeper pigment.

Named for Professor S. F. Light, of the University of California, who has not only materially aided these and other studies on the marine annelids of California, but also has given great impetus to studies of other marine invertebrates of the Pacific.

Distribution.—San Francisco Bay in the vicinity of Alameda (type) and west Berkeley beaches; also in the muddy bottom of Lake Merritt, Oakland, near its mouth into the estuary; associated with another species of *Eteone* described below (p. 131).

Systematic discussion.—*Eteone lighti* differs strikingly from other species of *Eteone* in the shape and proportions of its prostomium, the reduction of dorsal portion of first segment, the long cirriform anal cirri, the triangular, foliaceous dorsal cirri, and the ventrally directed ventral cirri.

Eteone dilatae sp. nov.

(figs. 40–42)

Diagnosis.—Length to 50 mm.; width 0.8 to 1.5 mm.; number of segments to 250; long, slender, cylindrical except for an anterior dilated region extending through about 20 to 25 segments posterior to the first few segments.

Prostomium elongate, trapezoidal, tapering anteriorly, its posterior border more than two and one-half times as long as anterior margin, a short anterior part slightly set off from the main part and having at sides 2 pairs of slender, subulate, subequal antennae which exceed in length the anterior end of prostomium (fig. 42). A pair of black eyes on posterior fifth of prostomium and a minute nuchal papilla at the posterior margin (fig. 42).

First segment a well-developed ring, dorsally about one and one-half times as long as segment 2, or shorter in some individuals (fig. 42), and ventrally produced in a conspicuous lower lip, provided laterally with 2 pairs of cirriform tentacles which are shorter than prostomial antennae.

Anterior parapodia short, deep, the dorsal and ventral cirri about equally large and long and surpassing the short but deep neuropodial lobe, which is provided with about 14 setae and an aciculum. Median parapodia (fig. 40) becoming larger and relatively conspicuous until almost as long as body is wide at segments 150–200; neuropodial lobes increasing in length posteriorly and coming to extend laterally well beyond distal end of ventral cirrus (figs. 40, 41), pre- and postsetal lobes subequal. Dorsal cirri subsymmetrical throughout, subrectangular but with a slightly inflated area at dorsal inner margin, this area slightly paler than rest of dorsal cirrus (figs. 40, 41); cirrophores low, flat.

Proboscis clavate when everted, smooth except for soft papillae on distal half. Anal cirri 2, thick, conical, together as wide as last segments and about twice as long as wide.

Color in life pale greenish yellow.

Distribution.—In sandy beaches at outer side of Bodega sand spit, Sonoma County (type); Marine View, San Mateo County, and Dillon Beach, Marin County, California.

Systematic discussion.—*Eteone dilatae* is most nearly related to the *E. longa* group in that it has symmetrical, more or less quadrangular, dorsal cirri and a well developed peristomial ring which lacks setae or parapodial papillae. It differs, however, from *E. longa* or any of its varieties in (1) the

shape and proportions of its prostomium, (2) the presence of an anterior dilated region of the body, (3) the subapical swelling of the dorsal cirri, and (4) the length of the neuropodial lobes in the median region. It is readily distinguished in the field because of its long slender form and its habitat, which is relatively clean sandy beaches.

Eteone californica sp. nov.

(figs. 43-46)

Diagnosis.—Length 10-20 mm.; width 0.7 to 1.0 mm.; number of segments 80 to 95. Prostomium broadly truncate, longer than broad (fig. 43), the 4 frontal antennae on an anterior part somewhat set off from the main part, cirriform, subequal, projecting laterally, about as long as anterior width of prostomium. Eyes 2, black, set near posterior margin of prostomium, separated by a distance equal to about one-third of width of prostomium. A minute nuchal papilla at posterior margin of prostomium (fig. 43).

Segment 1 almost twice as long as segment 2, well developed both dorsally and ventrally, with 2 pairs of cirriform tentacles which are somewhat longer than segment 1 (fig. 43); lower lip with 3 longitudinal grooves.

Parapodia well developed, with thick glandular bases and stout neuropodial lobes, extending laterally well beyond ventral cirri throughout (figs. 44, 45), the pre- and postsetal lips about equal anteriorly, the postsetal lip somewhat longer posteriorly (fig. 45). Dorsal cirri thick, inflated, subquadrangular, broader than long in mid-region, becoming as broad at about segment 66, and somewhat longer more posteriorly, never extending distally beyond neuropodial lobes. Setae relatively few (4 to 12) in a fascicle; articulation as in figure 46.

Anal cirri 2, thick, slightly clavate, twice as long as broad, their length equaling that of the last 3 segments, together broader than last body segments.

Color in life pale or ashy white, with light brown diffusions over cirri; brownish in preservative.

Locality.—San Francisco Bay (type), east side, in sandy mud flats; associated with *E. lighti* (p. 127).

Systematic discussion.—*Eteone californica* differs from *E. longa* (Fabricius) in that the prostomium has a nuchal papilla, the ventral cirri are conspicuously shorter and smaller and do not extend laterally to distal ends of the neuropodial lobes.

Eteone balboensis sp. nov.

(figs. 49-51)

Diagnosis.—Length about 40 mm.; width 2 mm.; number of segments to 125. Prostomium about three-fourths as long as wide, without eyes, but with a minute nuchal papilla at its median posterior border (fig. 49).

Dorsal cirri symmetrical, longer than broad, rectangular in median region (fig. 50), trapezoidal in posterior region (fig. 51). Ventral cirri bluntly conical, small throughout length; exceeded in length by the neuropodia except in first 10 to 15 segments; closely apposed to posterior face of neuropodia. Otherwise much like *E. californica* (p. 131).

Locality.—Balboa Bay, near Corona del Mar, California (type), in sandy mud beach.

Systematic discussion.—*E. balboensis* differs from *E. longa* in having no eyes and having ventral cirri which are minute. It is distinguishable from *E. californica* in lacking eyes and having dorsal cirri which are longer than broad throughout, these tapering somewhat distally in posterior region.

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TRYPANOSOMA NEOTOMAE, SP. NOV., IN
THE DUSKY-FOOTED WOOD RAT
AND THE WOOD RAT FLEA

BY
FAE DONAT WOOD

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TRYPANOSOMA NEOTOMAE, SP. NOV., IN THE DUSKY-FOOTED WOOD RAT AND THE WOOD RAT FLEA

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INTRODUCTION

IN THE COURSE of examining the blood of large numbers of dusky-footed wood rats, *Neotoma fuscipes annectens* Elliot and *N. f. macrotis* Thomas, in order to discover whether or not they were infected with *Trypanosoma cruzi* Chagas, the cause of American human trypanosomiasis (Wood, 1934), it was found that both subspecies harbor a trypanosome which is distinct from *T. cruzi*.

In morphology this haemoflagellate resembles *Trypanosoma lewisi* Kent, the common blood parasite of Norway rats (*Rattus norvegicus* Erxleben). However, there are important physiological and cyclical differences, upon the basis of which it has been named *Trypanosoma neotomae*. Its distinguishing characteristics are that it occurs naturally in the two subspecies of wood rats mentioned above, and in the wood rat flea *Orchopeas* (*Ceratophyllus*) *wickhami wickhami* Baker (identified for me by Professor M. A. Stewart, of The Rice Institute, Houston, Texas). It possesses a high degree of host specificity for wood rats, and it has not proved infective for Norway rats. Conversely, Portola wood rats (4 specimens) have failed to become infected after inoculation with *T. lewisi*. The incubation period is longer (more than 26 days) than in *T. lewisi*, which is usually 4 or 5 days. The duration of the infection with *T. neotomae* is usually several months—8 months in one specimen—whereas in 40 albino rats infected with *T. lewisi* (Marmorston-Gottesman, Perla, and Vorzimmer, 1930), the average duration was 27.4 days, none exceeding 35 days.

The life history of *T. neotomae* has been worked out by the writer, except for the method of multiplication in the vertebrate host. Because wood rats breed seasonally (Donat, 1933), and the young are difficult to rear under ordinary laboratory conditions, it was only rarely that young, nonimmune animals could be obtained for experimental inoculations.

The writer wishes to express appreciation to Professor Charles A. Kofoid for his helpful advice and constant interest in this problem.

MATERIAL

Sixty-one specimens of the Portola wood rat, *Neotoma fuscipes annectens*, have been trapped in the vicinity of Berkeley, California: 58 from Strawberry Canyon, 1 from Woolsey Canyon, 1 from Wild Cat Canyon, and 1 from the attic of a residence near Wild Cat Canyon; 12 of the 61 rats, or 19.6 per cent, carried a light infection with *T. neotomae*. One Portola wood rat from

Ben Lomond in the Santa Cruz Mountains showed no trypanosomes in its blood.

Seventy-seven specimens of the San Diego wood rat, *N. f. macrotis*, from San Diego County, California, proved to be negative for *T. neotomae*, but one from Los Angeles County, California, had an infection with this trypanosome which it retained for nine weeks, at which time the rat was sacrificed in an experiment.

The trypanosomes of these two subspecies of wood rats have been considered to be the same species for the following reasons: it has been possible to infect a San Diego wood rat with the trypanosome occurring in Portola wood rats; they are identical in morphology, in long persistence in the host's blood, in effect on the host, and neither has proved to be inoculable into Norway rats.

Approximately 200 fleas from Portola wood rats and 10 from San Diego wood rats were examined for trypanosomes. None of the fleas, *Orchopeas* (*Ceratophyllus*) *caedens caedens* Jordan (identified by Professor M. A. Stewart, of The Rice Institute, Houston, Texas), from the San Diego wood rats showed any flagellates in their digestive tracts. In every collection of fleas, *O. (Cerat.) wickhami wickhami* Baker, from infected Portola wood rats, some or all showed trypanosomes in their digestive tracts. One Portola wood rat which showed no trypanosomes in its blood, harbored a flea containing trypanosomes.

METHODS

The wood rats were examined by placing them in a cylinder of wire netting of $\frac{3}{8}$ -inch mesh, having a detachable cap. The rat was held securely in place by two small sticks thrust through the netting behind it. A drop or two of ear blood was mixed with a drop of sodium citrate solution and examined for trypanosomes. Stained smears also were made both of infected rat's blood and of the flagellates from the digestive tracts of the fleas.

TRYPANOSOMA NEOTOMAE IN THE WOOD RAT, NEOTOMA

Trypanosoma neotomae is a long, slender flagellate with a pointed, tapering posterior end (pl. 9, figs. 1, 5, 9). Its close resemblance to *T. lewisi* is very marked. For the strain of *T. lewisi* used for comparative studies the writer is indebted to Professor F. G. Novy, of the University of Michigan. The average length of 15 specimens of *T. neotomae* was 29.4μ , the extremes being 28 and 30μ . The average length of 15 adult specimens of a known strain of *T. lewisi* was 29.2μ , with a range of 25 to 31μ . In dry Giemsa-stained blood films the oval nucleus is in the anterior half of the body. The parabasal body is rodlike, but it may lie in such a position as to appear oval or lobed (pl. 9, figs. 5, 12, 13). In some specimens the flagellum may be seen to arise from a minute granule, the blepharoplast, just anterior to the parabasal. The free end of the flagellum is relatively short, being one-fourth or less the length of the body. The undulating membrane may vary slightly in width but is always narrow, with but few shallow undulations. Specimens of *T. neotomae* have been seen with a

rather large amount of cytoplasm surrounding the free end of the flagellum, giving the organism a thick, heavier appearance than *T. lewisi*. Also, more forms containing numerous granules in the cytoplasm have been seen than in *T. lewisi*. These have been described (Wenyon, 1926) in many trypanosomes as metabolic products (volutin) and are probably not of specific significance, since organisms both with and without granules occur in the same species. Both *T. neotomae* and *T. lewisi* in stained smears tend to be fixed in a characteristic curved, half-moon shape.

Both *T. neotomae* and *T. lewisi*, in the living state, have been seen to swim spirally across the field in counterclockwise, overlapping circles.

T. lewisi undergoes a multiplicative stage in the blood of the Norway rat during the first five or six days of the infection. At this time it is exceedingly polymorphic. This phenomenon has not been observed in wood rats naturally infected with *T. neotomae*. In these, presumably, the multiplicative period had passed. However, a trypanosome with a double parabasal and a short fibril which appeared to be a developing flagellum was seen in a Portola wood rat on the second day of its captivity (pl. 9, fig. 1). This is the only indication of division in the blood stream that has been observed.

EXPERIMENTAL ATTEMPTS TO INFECT WOOD RATS AND OTHER MAMMALS

Since no multiplication by *T. neotomae* had been observed in the peripheral or heart blood of naturally infected wood rats, various experimental animals were inoculated in order to determine, if possible, the complete course of the infection in the vertebrate host. The following animals were inoculated intraperitoneally with blood of naturally infected Portola wood rats: 19 young albino Norway rats, 1 young laboratory-bred, and 4 adult Portola wood rats, 3 young guinea pigs, 1 young rabbit, 1 kitten, 2 house mice, and 2 young white-footed mice (*Peromyscus* sp.). Examinations of the blood of these animals for periods of two weeks to one month after inoculation failed to reveal any trypanosomes.

At a later date another series of inoculations was carried out on San Diego wood rats. Three were inoculated with blood of naturally infected Portola wood rats. One of these (expt. 138), an adult female, was examined every two or three days from the 5th to the 26th day after inoculation, during which period it was negative for trypanosomes. When examined again on the fifty-fourth day its blood was found to contain numerous *T. neotomae*, that is, two or three per field, high dry objective and 6 × oculars being used. These were all of the slender "adult" type. Careful search failed to reveal any signs of division. Apparently the period of division had occurred sometime between the 26th and 54th days. The trypanosomes were last seen on the 58th day, the blood being negative when examined on the 61st and 66th days.

A second San Diego wood rat (expt. 139), born in the laboratory where it could not have acquired the infection, was inoculated at the age of 21 days. This rat was examined from the 5th to the 29th day. One trypanosome was

seen on the 23d day only. Examinations of its blood approximately two and five months after inoculation showed it to be still negative.

The third rat (expt. 148), a three-months-old female, also laboratory raised, failed to show any trypanosomes when examined from the 13th day after inoculation through a period of two months, careful examinations being performed every other day.

Seven other San Diego wood rats were inoculated with blood from infected rats of the same subspecies. Four of these did not show any trypanosomes in their blood although they were examined every two days throughout a period of more than two months.

One of the rats (expt. 149), a three-months-old, laboratory-raised female, was negative from the 6th to the 16th day, but showed a few trypanosomes from the 18th to the 21st day. These were of the slender adult type.

Another rat (expt. 154) in this group, a young female previously inoculated (expt. 148) from an infected Portola wood rat, with negative results, was inoculated and examined from the 1st day through nearly three months after inoculation. Trypanosomes were seen on the 2d and 3d days only. These were probably survivors from this second inoculation.

A third rat (expt. 152), a small, thin, adult female, showed a few trypanosomes from the 1st to the 63d day after inoculation. During the infection (38th day) this rat developed a large swelling on the right side of the nose at the base of the vibrissae. Within two days this opened and a large amount of pus exuded. The lesion was painted with iodine and within two weeks had healed completely. The weakened condition of this rat may account for the long persistence of the trypanosomes. No multiplication of the parasites was noted.

Since inoculations with infected blood did not reveal the method of multiplication in the mammal, two experiments were performed, infected fleas being used as the inoculum. An infected flea was teased up in saline and placed in a razor cut in the ear of a young albino Norway rat, thus introducing the trypanosomes directly into the blood stream. This rat was examined for one week (probably an insufficient period) and no trypanosomes appeared in the blood.

A Portola wood rat, which had been inoculated with infected blood three days previously (expt. 142), was fed the digestive tract of an infected flea, and the teased-up digestive tract of another infected flea was placed in its eye. Examinations over a period of seven weeks did not reveal any trypanosomes in the rat's blood.

In all the experiments except no. 138, in which trypanosomes appeared in the peripheral blood of the animal for varying periods after inoculation, it is probable that these trypanosomes represented the original organisms used in the inoculum, since they did not noticeably increase in number and no division was seen. In experiment no. 138, the relatively large number seen in the blood on the 54th day could not all have been included in the 1.5 cc. of citrated blood used as the inoculum. In the other experiments where trypanosomes were seen in the animal within a day or so after inoculation and the number remained fairly constant for some time before final disappearance, it is probable that

there existed in the blood a "reproduction-inhibiting antibody" as described by Taliaferro (1924) for *T. lewisi*, but not a complete immunity of a kind that would kill the trypanosomes at once. Both natural and acquired immunity were probably factors in the negative results of the inoculations of wood rats. In rats of unknown history there may have been a previous immunizing infection, or, as is probable in laboratory-bred rats, a natural immunity. Other factors influencing susceptibility are the kind of inoculum used and the method of inoculation. Perhaps a higher percentage of positive results might have been obtained if infected flea feces, rather than blood, had been used as inoculum.

EXAMINATIONS OF TISSUES

Since the trypanosomes appeared in the blood of some animals for several months and no division could be found, the possibility of multiplication within the host's tissues was considered. Heart, lung, brain, spleen, liver, and voluntary muscle of two infected Portola wood rats were sectioned and examined, but no trypanosomes, or anything that could be interpreted as stages of a trypanosome, were noted.

Later, an infected San Diego wood rat which had been in the laboratory for nine weeks was killed and its tissues sectioned, stained in Ehrlich's haematoxylin, and examined. Sections of thyroid, thymus, cerebrum, lumbar spinal cord, heart, hind leg muscle, bone marrow from ribs and femur, lungs, liver, pancreas, kidney, adrenals, peritoneum, stomach, small intestine, caecum, rectum, testis, and inguinal lymph nodes failed to show any multiplicative stages.

SYMPTOMATOLOGY

In this infection there seems to be a relatively well-balanced relationship between host and parasite, since the infection persists for a long time (8 months in one case and 2 to 6 in others), and the host appears to suffer no notable effects from the parasites. In none of the naturally infected rats have any symptoms occurred which could definitely be attributed to the trypanosomes.

In two cases (expts. 152 and 153) out of three, San Diego wood rats in which the same inoculum was injected intraperitoneally on the same day showed a paralysis of the hind legs and a peculiar deformation (a humping) of the lumbar vertebrae. This condition was first noted on the 2d day after inoculation and persisted as long as the animals were kept under observation (3 months). The paralysis gradually decreased in intensity, but the hunched condition did not improve. One of these 2 rats had been given a light experimental infection with *T. neotomae* previously and did not show any trypanosomes in its blood following this second inoculation. The other showed a light infection from the 1st to the 64th day after inoculation. Marmorston-Gottesman, Perla, and Vorzimmer (1930) report a similar paralytic condition in two out of forty albino rats injected intraperitoneally with *T. lewisi*. In their rats, the condition appeared in the second week and disappeared at the end of the third.

Before definitely attributing such symptoms to the trypanosomes, one should consider the possibility that other latent infections were stimulated by the inoculation. Dr. K. F. Meyer has informed the writer that wood rats are carriers of a neurovirus which is known to produce paralytic symptoms, so it is possible that the trypanosome injection is only indirectly the cause of the symptoms described above.

T. NEOTOMAE IN THE FLEA ORCHOPEAS

The observations upon developmental stages of *T. neotomae* in the Portola wood rat flea, *Orchopeas wickhami wickhami*, have been made in large part from living material and fixed, haematoxylin, or Giemsa-stained smears. It is assumed that this trypanosome is the insect phase of *T. neotomae*, since infected rats harbored infected fleas whereas uninfected rats usually harbored uninfected fleas.

Intracellular spheres of dividing trypanosomes in the stomach epithelium, as described for *T. lewisi* (Minchin and Thomson), have not been observed in fleas infected with *T. neotomae*. In a few stomachs, small numbers of stubby crithidias were found. These were also seen in the small intestine and rectum (pl. 9, fig. 8). Besides crithidias, the rectums and feces were often swarming with stubby trypanosomes (pl. 9, figs. 3, 4, 6, 7). These resemble closely the infective stages of *T. lewisi* in *Ceratophyllus fasciatus* (see Minchin and Thomson, 1915). In one digestive tract which was removed intact and examined in saline under a cover slip, there were large crithidias in the pyloric end of the stomach, a thick layer of crithidias (?) attached to the wall of the intestine, and numerous crithidias and trypanosomes in the rectum.

The writer attempted to raise wood rat fleas in the laboratory in order to obtain a clean stock to feed experimentally upon infected rats, for the purpose of studying the early stages of development in the flea. With this in mind, twelve fleas (*O. wickhami wickhami*) from four Portola wood rats were placed on an albino Norway rat which was in a large battery jar covered with muslin. The albino laboratory rat was used as host because its blood was known to be free from trypanosomes and its white fur and docile habits made collection of the fleas an easy task. At the end of seven weeks, no fleas were to be found on the rat, nor in the sand and debris on the floor of the cage, indicating that the Norway rat is not a favorable host for this species of flea.

One flea from a young Portola wood rat from Woolsey Canyon, Berkeley, showed numerous flagellates in the Malpighian tubules, intestine, and rectum when examined fresh in saline. This material, when teased up, fixed and stained in Jenner-Giemsa, showed enormous numbers of flagellates of the leptomonas type and some leishmaniform bodies with or without free flagella (pl. 9, figs. 2, 14). Careful search failed to reveal any crithidias or trypanosomes. For this reason, and since the host rat showed no trypanosomes in its blood, it is probable that this was an infection with *Leptomonas* sp.

INFECTION OF OTHER INSECTS

Besides the flea *Orchopeas*, Portola wood rats harbor in their nests and on their bodies numerous other parasites. The wood rat louse, *Neohaematopinus inornatus* Kellogg and Ferris (identified for me by Professor E. O. Essig), from wood rats infected with *Trypanosoma neotomae*, has been found by the writer to contain in its digestive tract trypanosomes morphologically like those in the rat's blood. Some forms which were either crithidias or degeneration stages were seen in the fresh, unfixed material.

Some unidentified mites have been numerous on the rats, but none of these has ever been found infected. In the nests and sometimes in the fur of the rats, there occurs a large flea, *Hystrihopsylla dippiei* Rothschild, also identified by Professor Essig. Eleven specimens of this flea, taken from wood rat nests in a locality where *T. neotomae* had been found in the rats, showed no trypanosomes in their digestive tracts. Ticks (probably *Ixodes ricinus californicus* Banks) have often been found on the rats. Their rôle as vectors of *T. neotomae* has not been ascertained.

Although the reduviid bug, *Triatoma protracta* Uhler, has not been found in wood rat nests in Berkeley where infection with *T. neotomae* is known to exist in the rats, specimens of this insect have been examined from Mount Diablo in Contra Costa County, from Lake County, Calaveras County, Riverside County, Los Angeles County, and San Diego County. None of these showed infection with *T. neotomae*, although the *Triatoma* from San Diego County were found to harbor *T. cruzi* (Wood, 1934). Attempts were made to determine whether these bugs could serve as vectors of *T. neotomae* by feeding them experimentally upon infected wood rats. Twenty-two specimens of *Triatoma* thus fed indicated that the blood forms remain in the stomach for at least twenty-three hours, after which they disappear. No trypanosomes were found in the feces of these bugs. It is interesting to note here that an adult *Triatoma* fed upon a rat heavily infected with *T. lewisi* failed to show any trypanosomes in its feces when examined twelve days later.

DISCUSSION

Trypanosoma neotomae belongs to a large group of rodent trypanosomes morphologically similar to *T. lewisi*. Those whose life histories have been studied show a type of development in fleas similar to that of *T. lewisi*. Wenyon (1926, vol. 1) lists 19 of these, examples of which are *Trypanosoma otospermophili* Wellman and Wherry from the California ground squirrel, *T. microti* Laveran and Pettit from the meadow mouse, and *T. duttoni* Thiroux from the house mouse. They have been given separate specific names, chiefly because attempts to inoculate them into Norway rats, the natural hosts of *T. lewisi*, have failed. Roudsky (1910) and Wenyon (1926) have suggested that perhaps the whole group of "lewisi-like" trypanosomes represent in reality only different strains of *T. lewisi*.

The whole problem seems to rest upon the timeworn question: What is a species? *T. lewisi* has, in the past, been considered a parasite of the Norway rat, inoculable into other animals with difficulty and only by special methods. If these wild rodent trypanosomes are, indeed, *T. lewisi*, they should be infective for Norway rats which have not been immunized by previous infection with *T. lewisi*. Since they are not, and in view of our present concept of *T. lewisi* as a species, there is justification for giving them separate specific names. Certainly, all the described members of this group seem to have become more or less permanently adapted or "fixed" physiologically to the hosts in which they were found. If we are willing to broaden our definition of *T. lewisi* so as to include trypanosomes of similar or identical morphology which develop in like manner in rodents and fleas, then we may properly class all these as *T. lewisi*.

In order to solve the question of specificity of this group of trypanosomes, the life histories of all should be studied, and cross inoculations of the different parasites made into different hosts. This would be a large task, if widely carried out. Perhaps in the meantime it is best to follow precedent and consider all trypanosomes of rodents which do not easily infect Norway rats as distinct from *T. lewisi*.

It is known that Strawberry Canyon, where most of the infected wood rats have been obtained, is also invaded by Norway rats, the natural hosts of *T. lewisi*. Because of the contacts of these two species, it is quite possible that there may be interchange of ectoparasites. Perhaps blood parasites may frequently be introduced by insect vectors from one host species to another. Norway rats and their parasites were introduced into America from the Old World; hence our New World rodents have been exposed to their diseases. It is therefore possible that *T. neotomae* and other similar trypanosomes of rodents are newly differentiated species derived from *T. lewisi*, but it is more probable that these infections have a longer history generally.

Incidentally, also, the flea host of *T. neotomae* bears very close resemblance to the common flea host of *T. lewisi*. So much so, in fact, that the writer at first considered them to be the same species.

SUMMARY

1. *Trypanosoma neotomae*, sp. nov., has been found in the blood of 12 out of 62 Portola wood rats (*Neotoma fuscipes annectens* Elliot) and 1 out of 78 San Diego wood rats (*N. f. macrotis* Thomas).

2. In size, behavior, and morphology in the wood rat it resembles *T. lewisi* of the Norway rat. Its development in the wood rat flea (*Orchopeas wickhami wickhami* Baker) is similar to that of *T. lewisi* in the common rat flea (*Ceratophyllus fasciatus* Bosc).

3. It is considered distinct from *T. lewisi* because: (a) Norway rats, the specific hosts of *T. lewisi*, have not proved susceptible to experimental infection with *T. neotomae* in 20 cases, (b) wood rats, in 4 cases, have proved refractory

to inoculation with *T. lewisi*, (c) *T. neotomae* has a longer incubation period and persists in the blood for a longer time than *T. lewisi*.

4. The following young laboratory animals have been inoculated with *T. neotomae* with negative results: 20 albino Norway rats, 3 guinea pigs, 1 rabbit, 1 kitten, 2 house mice, and 2 young white-footed mice (*Peromyscus* sp.).

5. Four adult Portola wood rats whose history of possible previous infection was unknown, and 1 young laboratory-bred Portola wood rat which had never been infected, were inoculated, with negative results.

6. Ten San Diego wood rats were inoculated with *T. neotomae*. Five were negative, 4 showed a few trypanosomes in their blood at periods varying from 1 to 63 days after inoculation, and one was negative up to 26 days but showed numerous *T. neotomae* when next examined on the 54th day, indicating that a multiplicative period had intervened.

7. Careful examinations of the blood and tissues of infected wood rats have failed to reveal the method of division of *T. neotomae*, although a Portola wood rat, shortly after capture, showed a trypanosome in its blood with a double parabasal and a cytoplasmic fibril resembling a secondary flagellum, indicating that multiplication is by binary fission in the blood stream.

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EXPLANATION OF PLATE 9

All figures drawn with the aid of the camera lucida. Magnification, $\times 3924$.

Figures 6, 7, 8 are from slides fixed in Schaudinn's fluid and stained in iron haematoxylin. All other figures are from dry-fixed smears, stained in Jenner-Giemsa.

Fig. 1. *Trypanosoma neotomae* from blood of a Portola wood rat. Note double parabasal and developing secondary flagellum.

Fig. 2. Dividing form of *Leptomonas* (†) from digestive tract of flea from Portola wood rat.

Figs. 3 and 4. Metacyclic *T. neotomae* from rectum of flea from infected Portola wood rat. Note fibrils in figure 3 connecting blepharoplast with parabasal body and nucleus. Note dark granule (blepharoplast †) embedded in parabasal body of figure 4.

Fig. 5. *T. neotomae* in blood of San Diego wood rat. Note two fibrils (rhizoplasts) connected to blepharoplast.

Figs. 6 and 7. Metacyclic *T. neotomae* from feces of flea from infected Portola wood rat.

Fig. 8. Crithidias of *T. neotomae*, from rectum of flea from Portola wood rat. One shows two parabasals.

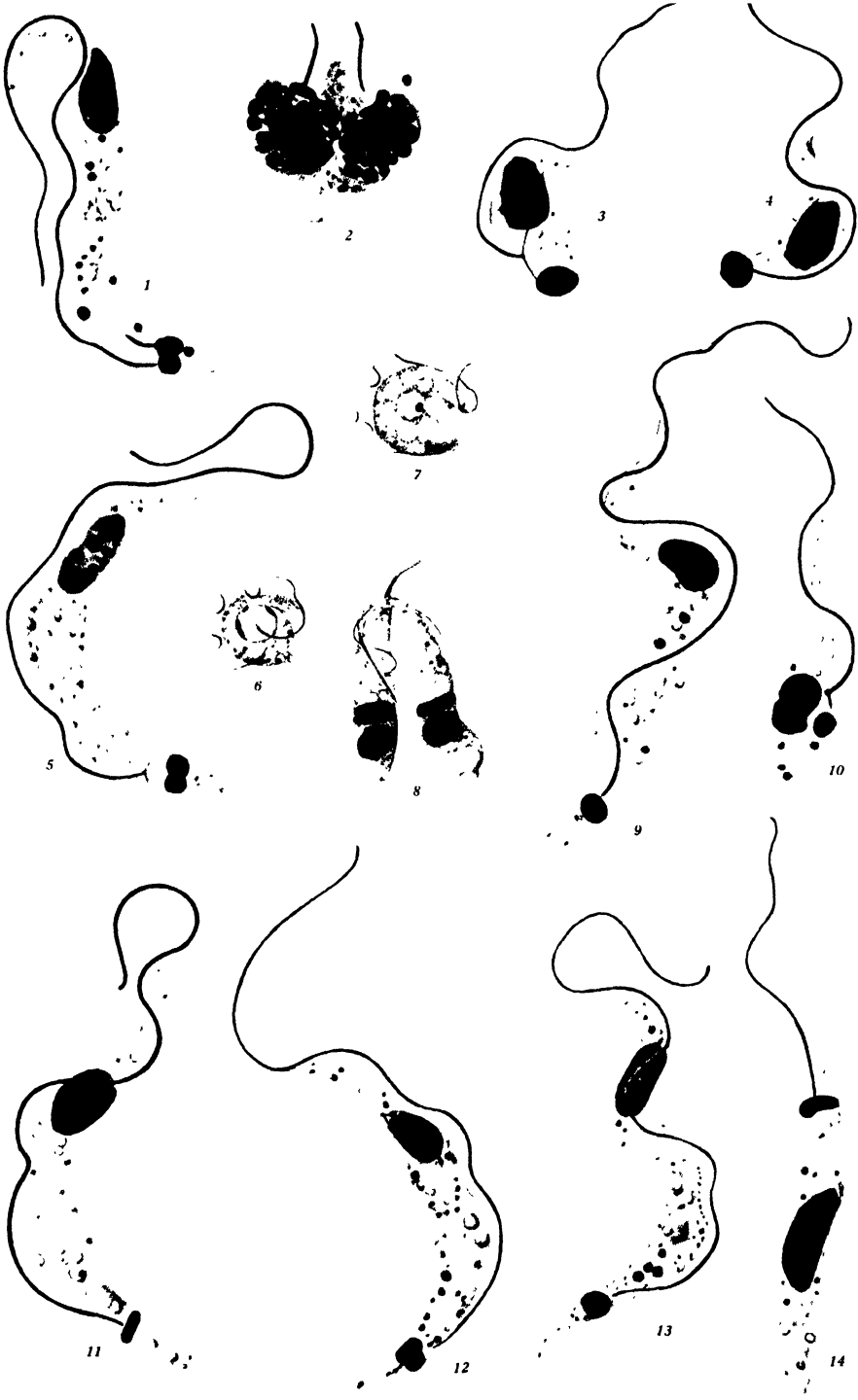
Fig. 9. *T. neotomae* from blood of Portola wood rat.

Fig. 10. Transition stage from crithidia to trypanosome, from rectum of flea from Portola wood rat infected with *T. neotomae*.

Fig. 11. *T. neotomae* from blood of Portola wood rat.

Figs. 12 and 13. *T. neotomae* from blood of San Diego wood rat.

Fig. 14. *Leptomonas* (†) from digestive tract of flea from Portola wood rat.



THREE NEW SPECIES OF
FRIDERICIA (ENCHYTRAEIDAE)
FROM CALIFORNIA

BY
A. W. BELL

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THREE NEW SPECIES OF FRIDERICIA (ENCHYTRAEIDAE) FROM CALIFORNIA

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THE ENCHYTRAEIDAE are small oligochaetes, dwelling in moist soil inland or along the shores of lakes and oceans. The terrestrial species may be found in almost any place where the soil is kept damp through the dry seasons. They are commonly collected from along canals and stream banks, around springs, under dripping faucets, about decaying wood, and under leafmold. They dwell in the surface stratum, where the plant food is abundant, breeding rapidly when conditions are optimum and disappearing when drought comes. They are easily collected and may be kept indefinitely on moist moss or on filter paper. They are simple in structure and most of them are transparent, factors which make them valuable for classroom demonstration.

In spite of their abundance and the number of species that remain to be identified, almost nothing has been done on the taxonomy of our California Enchytraeidae. The only investigations on the west-coast enchytraeid worms which have been made up to the present are those of Eisen (1905), who collected only in the seaports and adjacent regions, leaving the inland and mountainous areas as virgin fields for future workers. This paper presents descriptions of three new species of *Fridericias* together with a dichotomous key to the known species of this genus.

Fridericia sacculata, sp. nov. (plate 10, figs. 1-16)

Diagnosis.—Length 12-18 (17) mm.; diameter 0.5 mm.; number of somites 50-64 (59). Color pale gray to white, transparent. Prostomium rounded to blunt at tip. Head pore at 0/I. Dorsal pores begin in VII. Skin with 3-5 narrow, transverse rows of gland cells in anterior somites. Clitellum on XII and $\frac{1}{2}$ XIII, with 30 or more transverse rows of gland cells. Setae of *Fridericia* type, anteriorly 4-6 (rarely 2-3), posteriorly 4-2. Lymphocytes of 2 kinds: larger ones nucleated, ellipsoidal, with coarsely granular cytoplasm; smaller ones nonnucleated, ovoid, of homogeneous structure. Dorsal vessel arises as far back as XXII/XXIII. Brain, one and one-half times as long as broad, anteriorly convex, posteriorly truncate or slightly emarginate, convergent cephalad. Peptonephridia tubular with several branches in V. Intestine with chylus cells in XV, XVI, and XVII. Nephridium with post-septal about twice as long as anteseptal, duct arising just behind septum. Large sperm sac formed by septum X/XI's being forced forward. Sperm funnel about three times as long as broad, sperm duct long and much coiled. Penial bulb large, of Lumbricillid type. Spermatheca: large ampulla with 7 or more diverticula at base, communicating separately with oesophagus near middorsal line in V; ectal duct about three times as long as ampulla; 2 ectal glands at IV/V.

Remarks.—Described from 17 living and 2 sectioned specimens, besides numerous unrecorded observations on unlimited material available. Sectioned specimens in possession of author; cotypes deposited in the United States National Museum, Washington, D. C.

Collected from the surface soil under the decaying leafmold beneath the bald cypress, *Taxodium distichum*, just south of the Life Sciences Building, University of California, Berkeley.

Affinities.—This species belongs to that group of species which has a number of spermathecal diverticula ranging around 7, setae from 4 to 6 per fascicle, and segments reaching from 60 to 65 in number. It is almost identical with *F. galba* Hoffmeister; the latter, however, possesses only 3 to 5 spermathecal diverticula and lacks the sperm sac. It is similar to *F. udei* Bretscher, except for the possession of 1 or 2 more spermathecal diverticula, a few more segments, and the sperm sac. Another close relative is *F. antarctica* Beddard, which differs, however, in the body color, in the origin of the dorsal vessel, and in the absence of the sperm sac.

EXTERNAL CHARACTERS

The body is slender, being about 0.48 mm. in diameter preceding and following the clitellum, which is but slightly enlarged in the most mature worms. The length varies according to age, the maximum being about 18 mm. The number of segments also varies with maturity, the maximum so far observed being 64. The body is at first grayish, but if the worms are kept on moist filter paper and the intestinal contents eliminated, they become white and quite transparent. The prostomium is blunt, being either rounded or slightly flattened at the anterior end. The skin is glandular in the anterior 7 or 8 segments and occasionally beyond the clitellum, there being 3 to 5 transverse rows of cells. Also, the skin of the prostomium and the first few somites (pl. 10, fig. 4) has a number of fine papillae. The clitellum, situated on the 12th somite and for some 5 rows of gland cells beyond the setae in the 13th, is but slightly elevated above the level of the other segments, and in many specimens shows no elevation whatever (pl. 10, fig. 16). The head pore (pl. 10, fig. 4) is a longitudinal slit in the intersegment 0/I. The first dorsal pore is in VII. Setae are of the *Fridericia* type anteriorly (pl. 10, fig. 2), but posteriorly many fascicles are found in which all 4 setae are of equal length (pl. 10, fig. 1). The longest setae measure about 75μ in length.

INTERNAL CHARACTERS

Nervous system.—The brain (pl. 10, fig. 3) is in I and II. It is rounded anteriorly, slightly convex to truncate posteriorly, and measures approximately 150μ long by 100μ wide. The subpharyngeal ganglia and those immediately following it are typically enlarged. There are large ventral swellings in XII, XIII, XIV, which in serial sections prove to be glands lying alongside the ganglia and connecting with the skin on the midventral side.

Digestive tract.—The peptonephridia (pl. 10, fig. 7), which arise in IV and extend into V, are cylindrical sacs with several branches in V, some of which are secondarily bifurcated. Chlorogog cells begin in V and are lacking in XII. Chylus cells (pl. 10, fig. 10) are present in XV, XVI, and XVII. They are abundant in number and are bounded at their inner margins by flattened

ental epithelial cells. The intracellular canals are ciliated and run the entire length of the cell, coiling around the nucleus and making a half turn at the base of the cell. Interstitial cells may lie upon the surface of the chylus cell.

Circulatory system.—The dorsal vessel arises at the intersegment XXII/XXIII in the largest specimens. One worm was found with the origin in the anterior end of XXIII. In studying a large number of worms, the writer has noted that the dorsal vessel recedes with the increase in the number of segments. A small cardiac gland is present in the dorsal vessel. There are four pairs of symmetrical aortic arches (pl. 10, fig. 5) in the general region of III, IV, and V. Both nucleated and nonnucleated lymphocytes (pl. 10, fig. 12) are present. The former are large, roughly elliptical when viewed from the flat surface (30μ by 40μ), and possess large granules; the latter are small (5μ by 10μ), ovoidal, and of homogeneous consistency.

Excretory system.—The nephridium (pl. 10, fig. 6) consists of an anteseptal with a protruding ciliated nephrostome, a postseptal about twice as long as the anteseptal, and a short duct arising from the postseptal just behind the septum.

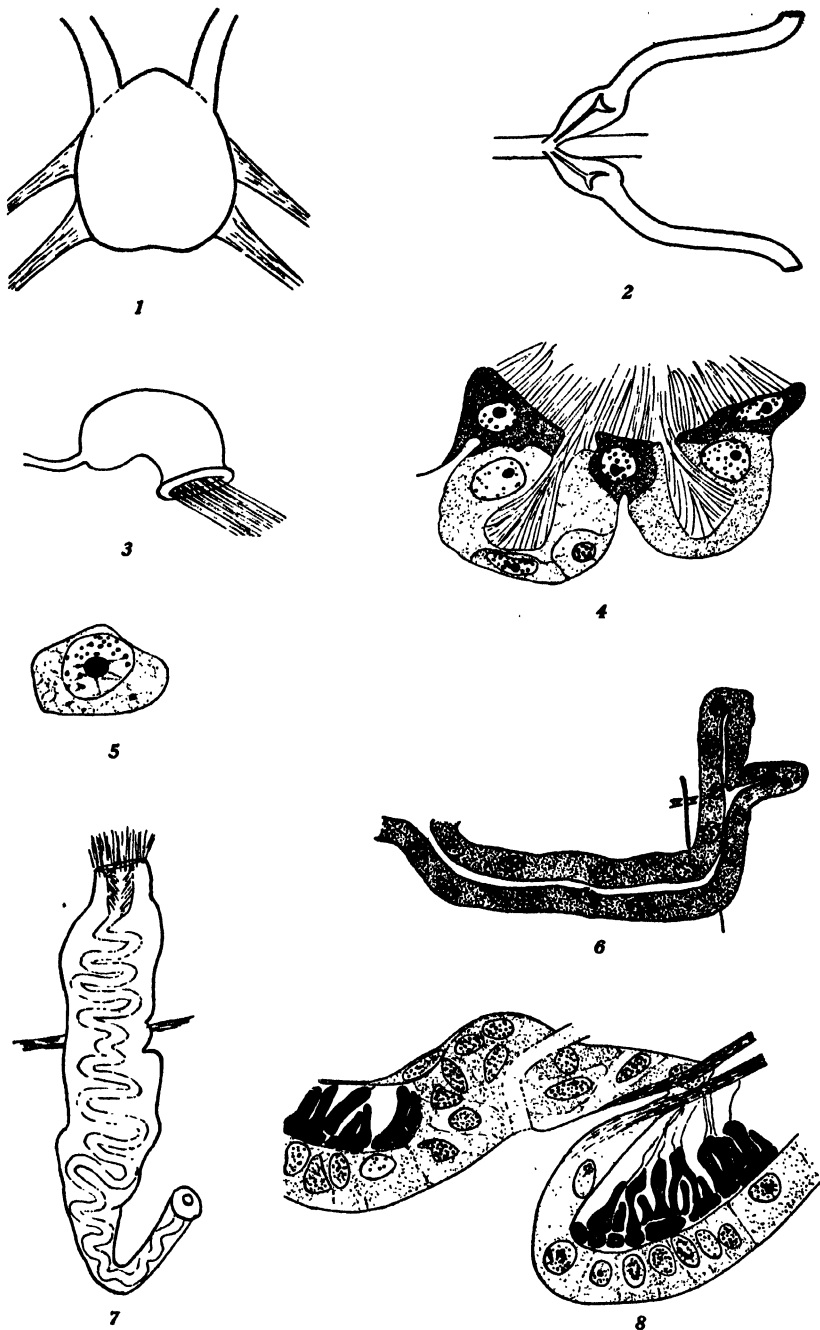
Reproductive system.—The testes lie at the base of the septum anteriorly in XI; the ovaries lie in the same position in XII. Upon maturing, the germ cells are produced in prodigious quantities, forcing the septa out to accommodate them (pl. 10, fig. 16). In some large worms the septum X/XI is forced as far forward as VIII/IX, taking the septum IX/X forward with it. It is this outstanding characteristic that prompted the name of *F. sacculata*. A slight pressure will cause exudation of sperm through the dorsal pore in XI. The ovaries also produce large numbers of egg masses ("Teilovaria"), which force the septum XII/XIII back into a kind of egg sac. Also, egg masses may be found in other segments, even as far back as somite XX. The spermathecae (pl. 10, figs. 9 and 15) are large, consisting of a long ectal duct with 2 glands at the orifice at IV/V (pl. 10, figs. 9 and 12) and a broad ampulla with 7 or 8 diverticula at the base. The diverticula are frequently bipartite (pl. 10, fig. 9). The ampullae unite separately with the oesophagus near the middorsal line in V. The penial bulb (pl. 10, fig. 14) is of the Lumbricillid type, with but one tier of gland cells, some opening into the vas deferens, others opening upon the surface of the bulb. Under the slight pressure of the cover slip the penis may be forced out (pl. 10, fig. 11); it is about 30μ long, and 13μ wide at its greatest diameter.

Septal glands are present in the usual place for these structures, that is, in IV/V, V/VI, and VI/VII. Observed from the side, they present a lobulated appearance (pl. 10, fig. 9).

Fridericia berkeleyensis, sp. nov.

(Text figs. 1-8)

Diagnosis.—Length 4.5-7 mm.; diameter 0.2 mm.; number of somites 33-43. Color whitish and transparent. Prostomium rounded. Head pore 0/I. First dorsal pore in VII. Skin with a single row of transversely arranged gland cells behind each row of setae. Except in II-VI, segments appear to be doubled externally. Clitellum in XII and $\frac{1}{2}$ XIII, slightly elevated,



Figures 1-8. *Fridericia berkeleyensis*, sp. nov.

Fig. 1. The brain. From life, $\times 200$.

Fig. 2. The spermathecae, dorsal aspect. From life, $\times 200$.

Fig. 3. The sperm funnel, with sperm protruding. From life, $\times 400$.

Fig. 4. Chylus cells with adjacent ental epithelial cells and interstitial cells. From serial sections, $\times 1100$.

Fig. 5. Small lymphocyte from coelom. From serial sections, $\times 1970$.

Fig. 6. Peptonephridium. Reconstructed from serial sections, $\times 666$.

Fig. 7. Nephridium. From life, $\times 400$.

Fig. 8. Penial bulb. From cross-section, $\times 1100$.

not very glandular. Setae, 2 per fascicle. Lymphocytes nucleate and nonnucleate, small and sparse. Dorsal vessel arises between XVII and XX. Brain about one and one-fourth times as long as broad, anteriorly projected forward, posteriorly emarginate, perfectly heart-shaped. Peptonephridia small, saclike, with a single bifurcation in V. Septal glands normal in position and number. Nephridium with anteseptal nearly equal to the postseptal; duct terminal. Spermathecae with ampullae devoid of diverticula; ental communications with the oesophagus nearly together in the middorsal region in V; no ectal glands. Spermm funnel about twice as long as broad; collar with narrow rim but large orifice. Vas deferens short and unmodified. Penial bulb small, of Lumbricillid type.

Remarks.—Described from 3 living and 2 sectioned specimens; unrecorded observations made upon several others. The specimens were collected under the bald cypress just south of the Life Sciences Building, University of California, Berkeley, California, in association with *F. sacculata*, described above. Sectioned specimens in the possession of the author; cotypes deposited in the United States National Museum, Washington, D. C.

Affinities.—This species belongs to that group of small bisetous worms which have simple spermathecal ampullae devoid of diverticula. The nearest related species is *F. bretscheri* Southern (see Southern, 1907, and Bretscher, 1902). A pronounced difference is found, however, in the shape of the brain, *F. bretscheri* having a brain that is two to three times as long as broad, and convex posteriorly. There is a slight difference also in the origin of the dorsal vessel: in Bretscher's species it is two to three segments anterior to that in ours. *F. bretscheri* also has an ectal gland, whereas ours has none. Finally, the postseptal of *F. bretscheri* is two to three times as long as the anteseptal; in ours they are of nearly equal size. Otherwise, the two species are quite similar.

EXTERNAL CHARACTERS

The species here described is very small, being but 0.2 mm. in diameter and 5–7 mm. long. The somites range from 33 to 43 in number. The worms are whitish and transparent. The prostomium is rounded. Frequently one is struck by the apparent duplicity of segmentation, except in segments II–VI, which is a result of swellings of the body wall between the intersegmental grooves and the setae. An incomplete, transverse row of gland cells is present in each segment just behind the setae. The clitellum, on XII and $\frac{1}{2}$ XIII, is not pronounced or very glandular. The setae number 2 per fascicle, except in I, XII, and the last segment, where they are lacking in all mature members of this genus.

INTERNAL CHARACTERS

Nervous system.—The brain (fig. 1) is the outstanding characteristic of this species. It is relatively very large for the size of the worm (100 μ long and 80 μ wide) and is perfectly heart-shaped.

Digestive tract.—The peptonephridia (fig. 6) are simple, saclike tubes arising in IV and passing back to the septum, whence they course dorsad in V, bifurcating near the terminus. The oesophagus is small, enlarging to the full size of the intestine in XIII. Chylus cells are present in XIII and XIV (in one specimen in $\frac{1}{2}$ XII to $\frac{1}{2}$ XIV). They have a remarkable shape, the

cavity often appearing like an Ehrlenmeyer flask with a broad base (fig. 4); that is, there is an intracellular sac, instead of the usual intracellular canal, which is ciliated throughout. There are but 6 chylus cells in cross section, and when the series of sections is studied it is seen that these unique cells are arranged in longitudinal rows, separated by flat or wedge-shaped ental epithelial cells. Interstitial cells are found upon the surface of the chylus cells. Chlorogog tissue is not well developed and does not exhibit the great display of granules found in so many other species of this genus.

Circulatory system.—The dorsal vessel arises in XX in the most mature specimens. As mentioned above for *F. sacculata*, the origin varies progressively with the length and the number of somites. In a 5-mm., 36-somite worm, for example, the dorsal vessel arises in XVII; in a 7-mm., 43-somite worm it arises in XX. A small cardiac gland is present. The blood is colorless and without circulating cells. The body cavity has but few lymphocytes, these being of the two usual types: the larger are nucleated, and elliptical when viewed from the flat surface; the smaller are of the nonnucleated, ovoidal variety. A third type of cell, found in the coelom of a sectioned and stained worm, gives the appearance of a phagocyte (fig. 5).

Excretory system.—The nephridium (fig. 7) consists of a large anteseptal without the usual projecting lip, a postseptal of approximately the same size, and a short terminal duct. The nephrostome is somewhat cylindrical and ciliated, with a broad orifice entering the coiled canal, the latter twisting back and forth in the anteseptal as well as in the postseptal. The excretory duct has no ectal swelling.

Reproductive system.—At first, the size of these tiny worms caused a suspicion that they were merely immature forms of the other species, *F. sacculata*, with which they were collected. The presence of mature spermatozoa in XI, however, convinced the writer of their maturity. Essential structural differences also eliminated the possibility of any kinship. The sperm funnel (fig. 3) is about twice as long as wide; its collar is narrow, but the orifice is large. The vas deferens is relatively short and without glands or swellings before entering the penial bulb. The latter (fig. 8) is quite small and seems not to be provided with the usual covering of muscle. The spermathecae (fig. 2) are simple in structure, the ampullae lacking diverticula. The ectal duct is about twice the length of the ampulla and does not possess discernible glands at IV/V. The ampullae narrow gradually toward the oesophagus, with which they communicate separately near the middorsal line in V.

Fridericia losangelensis, sp. nov.

(pl. 11, figs. 17-31)

Diagnosis.—Length, up to 13 mm.; diameter, up to 0.4 mm.; number of segments 48-53. Color whitish, transparent. Prostomium rounded. Head pore 0/I. First dorsal pore VII. Skin with indistinct transverse rows of gland cells. Clitellum well developed, gland cells not in transverse rows. Number of setae 4 (rarely 5) per fascicle anteriorly, down to 2 posteriorly. Dorsal vessel in XIX, with cardiac gland. Lymphocytes: large ones nucleated, nongranular, ellipsoidal; small ones nonnucleated, nongranular, ovoidal. Brain about 1.4 times

as long as broad, anteriorly and posteriorly convex, converging cephalad. Peptonephridia saclike, tuberculate, with a single tubular branch in V. Septal glands in usual place. Nephridial anteseptal large, about two-thirds the size of postseptal; excretory duct subterminal. Spermathecae with ampullae lacking diverticula; ental ducts unite to communicate with oesophagus by a single fine canal middorsally in V; no ectal glands. Sperm funnel about twice as long as broad. Penial bulb of Lumbricillid type, with 2 tiers of gland cells.

Remarks.—Described from observations on unlimited numbers of living worms, 4 of which were studied in detail and their structures recorded, and from studies of worms sectioned serially, both transversely and longitudinally. The characteristics of spermathecae, peptonephridia, and dorsal vessel were used in selecting this species from its habitat associates not of the same species.

Collected from the lawns and beneath the two large *Araucaria* trees near the entrance to the campus of the Los Angeles Junior College. Serial sections of specimens in the possession of the author; cotypes deposited with the United States National Museum, Washington, D. C.

Affinities.—This species belongs definitely to the *bulbosa* group, but is longer and has more somites than *F. bulbosa* Rosa, *F. rosae* Cognetti, *F. variata* Bretscher, *F. bulbifera* Friend, and others nearly related. It closely resembles *F. parva* Moore, but differs in the number of segments and in the shape of the spermathecal ampulla, the peptonephridium, and the brain. The shape of the peptonephridium sets our species apart from its nearest relatives.

EXTERNAL CHARACTERS

The body is from 10 to 13 mm. in length in mature living worms. Fixation reduces the length by about 25 per cent, a factor that should be taken into consideration in all measurements recorded from fixed worms. The diameter is from 0.36 to 0.4 mm. The number of segments in mature worms varies from 48 to 53. The color is white and the body wall is more or less transparent. The skin possesses indistinct transverse rows of glandular cells. There is a special area of gland cells around the ectal openings of the spermathecae, evidently reproductive in function. The clitellum, on XII and $\frac{1}{2}$ XIII, is well developed and glandular, the gland cells being heterogeneously arranged (pl. 11, fig. 29) and the small clear spaces between them occurring at random. The setae (pl. 11, fig. 20) are 4 in number, an occasional specimen being found with one or two pairs of ventral fascicles containing 5, a fact which is interpreted as a failure to shed a seta after new ones have grown in. A typical record of setae in a worm of 49 segments gives the following counts: 4 (rarely 3 or 5) per fascicle to XXIII; 3 laterally, 2 ventrally, from XXIV to XXVIII; 2 per fascicle to XLVIII, inclusively.

INTERNAL CHARACTERS

Nervous system.—The brain (pl. 11, fig. 18) measures 100μ wide by 140μ long; it is broad and slightly convex at both ends. In shape it is a rough oval, the sides converging a little cephalad.

Digestive tract.—The oesophagus enlarges gradually, reaching its greatest size in the postclitellar region. Chlorogog cells (pl. 11, fig. 27) begin in VI

and continue throughout the length of the intestine except for their absence in XII. They are large and abundantly supplied with large chlorogog granules. The chylus cells (pl. 11, figs. 21 and 26) are numerous in XIV, XV, and XVI, occasionally reaching a few cells forward into XIII. Their intracellular canals apparently do not possess cilia. The ental epithelial cells are elevated between the chylus cells and are covered with cilia. The peptonephridia (pl. 11, fig. 30) are long, tuberculate sacs, reaching from the beginning of IV to the end of V. From the terminus of the sac a slender branch passes straight dorsad, just under the body wall and lateral to the spermathecae.

Circulatory system.—The dorsal vessel arises in XIX in the largest specimens. In this species, also, there is a definite correlation between the length, the number of segments, and the origin of the dorsal vessel. For example, in 2 worms of 49 segments each, the dorsal vessel arose in XVIII, whereas in 2 other worms of 52 and 53 segments, respectively, the dorsal vessel arose in XIX. A cardiac gland is well developed (pl. 11, fig. 27). This is emphasized because Eisen (1905) has stated that the cardiac body is absent in the genus *Fridericia*. Its structure is somewhat like that of overlying chlorogog cells. The blood is colorless and does not contain any circulating cells. Coelomic corpuscles are of the two typical varieties (pl. 11, fig. 23): the larger ones are nucleated, from round to elliptical, and with few or no granules; the smaller ones are nonnucleated, ovoid, and of homogeneous consistency.

Excretory system.—The nephridia (pl. 11, fig. 19) consist of a large anteseptal with a ciliated nephrostome projecting forward, a postseptal about one and one-half times as large as the anteseptal, and an excretory duct that folds back from the terminus of the postseptal to give the appearance of arising from the middle of the postseptal. Measurements of living anteseptals by camera lucida prove that this structure may vary in length; it may stretch out or contract, so that the general volume is a more accurate criterion of relationship to the postseptal than is the length.

Reproductive system.—The testes and ovaries lie in the usual position. The sperm funnel is about twice as long as broad in the living state. Figure 28 is drawn from a longitudinal section and is shortened more than in life. It demonstrates the granular and smooth glandular cells that make up this interesting organ. Sperm are protruding from its orifice. The penial bulb (pl. 11, fig. 31) is of the Lumbricillid type, provided with both an encapsulating "ejector" muscle and a retractor muscle. The former is undoubtedly used to force the penis out, the latter to retract it. The gland cells are arranged in two tiers, the outer secreting upon the surface of the bulb, the inner into the vas deferens. The spermathecae (pl. 11, fig. 22) are bulbous, with a commissure between the two, connecting with the oesophagus dorsally in V by a single small canal, 2μ in diameter. When contracted in fixed specimens, the middorsal commissure is enlarged into a sac (pl. 11, figs. 24, 25). The ectal duct is about three times as long as the bulbous ampulla; the ental duct but slightly longer. There are no ectal glands.

A DICHOTOMOUS KEY TO THE KNOWN SPECIES OF THE GENUS
*FRIDERICIA**

- 1a. Worms possessing only the ventral fascicles of setae. (Formerly put as separate genus, *Distichopus*) *F. silvestris* (Leidy)
- b. Worms possessing both the ventral and the lateral fascicles of setae 2
- 2a. Worms with spermathecae lacking diverticula 3
- b. Worms with spermathecae possessing diverticula 30
- 3a. Worms with 2 (rarely 3) setae per fascicle 4
- b. Worms with 4 or more setae per fascicle 10
- 4a. Segments fewer than 50 5
- b. Segments more than 50 6
- 5a. Brain 2 to 3 times longer than broad; postseptal 2 to 3 times longer than anteseptal; ectal glands present *F. bretscheri* Southern
- b. Brain $1\frac{1}{4}$ times as long as broad; postseptal not much longer than anteseptal; no ectal glands *F. berkeleyensis*, sp. nov.
- 6a. Spermathecae unite with oesophagus in VI *F. pulchra* Friend
- b. Spermathecae unite with oesophagus in V 7
- 7a. Dorsal pores begin in VII 8
- b. Dorsal pores begin anterior to VII 9
- 8a. Segments 60–70; spermathecae bulblike; peptonephridia with bladder-like projections from the posterior enlargement, some of which may be prolonged into narrow diverticula or branches; no sperm sac mentioned *F. perigrinabunda* Michaelsen
- b. Segments about 56; spermathecae acorn-shaped; peptonephridia simple, bifurcated at posterior end, one branch narrow and short; single sperm sac in X communicating with XI *F. bollonsi* Benham
- 9a. Dorsal pores begin in V; segments about 55 *F. obtusa* Friend
- b. Dorsal pores begin in VI; segments about 64 *F. carmichaeli* Stephenson
- 10a. Setae as many as 4 (rarely 5) per fascicle (see note to 16b, below) 11
- b. Setae as many as 6 per fascicle or more 19
- 11a. Segments more than 54 12
- b. Segments fewer than 54 13
- 12a. Segments 54–60; length 20–28 mm.; diam. 0.7–0.8 mm.; peptonephridia massive, with many short tubular branches which may be secondarily ramified; blood a rosy tint *F. ilvana* Issel
- b. Segments about 65; length 18 mm.; diam. 0.5 mm.; peptonephridia with tubular main stem and 4–6 small tubular branches at end (i.e., like a glove); blood colorless *F. fuchsi* Eisen
- 13a. Peptonephridia saclike, with tuberculate surface or weakly branched at end 14
- b. Peptonephridia simple, without forking or branching 17
- 14a. Peptonephridia thick and compact, with free end frayed; brain posteriorly concave *F. johnsoni* Eisen
- b. Peptonephridia simple sacs, only slightly branched or bifurcated at end; brain posteriorly convex 15
- 15a. Segments as many as 53; peptonephridia tuberculate, with a single long branch at posterior end *F. losangelensis*, sp. nov.
- b. Segments as many as 45; peptonephridia branched or bifurcated at extremities ... 16
- 16a. Length 4–8 mm.; segments 32–42 *F. bulbosa* Rosa
- b. Length 8–10 mm.; segments 35–45 *F. rosae* Cognetti

[NOTE: these two forms are probably the same species. Stephenson (1923) describes *F. bulbosa* as having as many as 46 segments and measuring 4–15 mm. Southern (1907) describes *F. bulbosa* as having as many as 6 setae per fascicle.]

* Since the author submitted this paper for publication, there has come to his attention a description of a new species, *Fridericia pseudoargentea* Knöllner (Zool. Jahrb. Abt. f. Syst., Ökol. und Geog. der Tiere, 66:425–512), which is a small, bisetous worm with eight sessile diverticula upon the spermathecal ampulla. These characteristics place the species under 71 of the present key.

- 17a. Length 5-10 mm.; segments 40 or fewer; setae 4 per fascicle up to XVI, then 2 per fascicle to anus..... 18
 b. Length 12-15 mm.; segments 46; setae 4 per fascicle to XXV, then 2 per fascicle to anus..... *F. parva* Moore
- 18a. Length 5-6 mm. (rarely 8 mm.); segments 30-35 (rarely 40); brain concave anteriorly..... *F. glandifera* Friend
 b. Length up to 10 mm.; segments 35-40; brain egg-shaped..... *F. bulbifera* Friend
- 19a. Ampullae of spermathecae not attached to oesophagus..... *F. sonorae* Eisen
 b. Ampullae of spermathecae attached to oesophagus..... 20
- 20a. Length not more than 12 mm.; setae 4-6 per fascicle at most..... 21
 b. Length 13-25 mm.; setae usually more than 6 in larger fascicles..... 24
- 21a. Segments 41 or fewer..... 22
 b. Segments 50..... *F. alpina* Bretscher
- 22a. Length 11 mm.; spermathecal ampullae stand vertically above junction with oesophagus; mottled brown in color..... *F. praetoriana* Stephenson
 b. Length up to 6 mm.; spermathecal ampullae not elevated vertically; color whitish. 23
- 23a. Segments 27; spermathecae open by common passage into oesophagus at middorsal line. (Like *F. bulbosa* except for number of setae and spermathecae. See no. 16 above.)..... *F. variata* Bretscher
 b. Segments 40; spermathecae connect with oesophagus separately near middorsal line..... *F. harrimani* Eisen
- 24a. Segments 50 or fewer; brain anteriorly concave..... 25
 b. Segments more than 50; brain anteriorly convex..... 28
- 25a. Setae 7-10 per fascicle anteriorly and 4-7 posteriorly; brain indented both anteriorly and posteriorly. (Some structures inadequately described.)
F. stewarti Stephenson
 b. Setae 6-8 (seldom 9); brain convex posteriorly..... 26
- 26a. Peptonephridia saclike with tufts of many branches at their ends; excretory duct leaves postseptal at terminus..... *F. valdensis* Issel
 b. Peptonephridia slightly branched; excretory duct leaves postseptal just behind septum..... 27
- 27a. Setae as many as 9 per fascicle; brain slightly concave anteriorly; dorsal vessel arises in XVI..... *F. striata* (Levinson)
 b. Setae not more than 7; brain normal in shape; dorsal vessel arises in XX
F. bollonsi var. *oliveri* Benham
- 28a. Segments as many as 66; peptonephridia constituted of fascicles of tubules or ramified from a point; blood yellow-green..... *F. tusca* Dequal
 b. Segments as many as 58; peptonephridia very slightly branched or simple sacs; blood colorless..... 29
- 29a. Peptonephridia saclike, may be simple or with 2-3 short branches at ends; first dorsal pores in VI..... *F. Alba* Moore
 b. Peptonephridia simple sacs, no branches; first dorsal pores in VII.... *F. sima* Welch
- 30a. Each spermatheca with a single diverticulum..... 31
 b. Each spermatheca with 2 or more diverticula (see note under 31a)..... 32
- 31a. Ampullae of spermathecae united above, one communication with the oesophagus; length of worm up to 20 mm.; segments as many as 60..... *F. connata* Bretscher
 [Note: Southern (1907) gives 2 diverticula for each ampulla in this species.]
 b. Ampullae communicate with the oesophagus separately; length up to 8 mm.; segments as many as 36..... *F. monopera* Cognetti
- 32a. Spermatheca with 2 diverticula only..... 33
 b. Spermatheca with more than 2 diverticula..... 62
- 33a. Ampullae of spermathecae united into spherical sac, which communicates with oesophagus in middorsal line..... *F. gamothecae* Issel
 b. Ampullae of spermathecae not united as described above..... 34

- 34a. Setae 2 per fascicle (rarely 3) 35
 b. Setae 4 or more per fascicle 41
- 35a. Spermathecae without typical ampullae, the 2 diverticula lying as sessile pouches upon the duct; anteseptal of nephridium as long as the postseptal
F. biglobulata Bretscher
- b. Spermathecae with typical ampullae, from which the diverticula arise; anteseptal smaller than the postseptal 36
- 36a. Length up to 20 mm.; segments as many as 60 37
 b. Length up to 13 mm. only; segments not more than 55 38
- 37a. Peptonephridia long, slender, weakly branched at end; spermathecae rounded at end (no union with oesophagus mentioned) *F. bichaeta* Nusbaum
 b. Peptonephridia saclike, unbranched or weakly branched at end; spermathecae communicate with oesophagus at ental end *F. bisetosa* (Levinson)
- 38a. Length 7–10 mm.; segments 29–46 39
 b. Length 10–13 mm.; maximum segments range between 50 and 55 40
- 39a. Length 8–10 mm.; segments 41–46; diverticula of spermathecal ampullae straight and globular; skin glands uncolored; enucleate coelomic corpuscles 8–15 μ long
F. paroniana Issel
- b. Length 7–9 mm.; segments 29–41; diverticula of spermathecal ampullae cylindrical and curved inward toward duct in a spiral; skin glands with brown pigment; enucleate corpuscles 3–6 μ long *F. maculata* Issel
- 40a. Length 10 mm.; segments about 50; dorsal vessel arises in XXI; clitellum with glands in transverse rows *F. diachaeta* Bretscher
 b. Length 10–13 mm.; segments as many as 55; dorsal vessel arises in XX; clitellar glands not in transverse rows, irregular interstices between the gland cells
F. clitellaris Bretscher
- 41a. Fascicles with 4 (rarely 5) setae 42
 b. Fascicles with 6 or more setae 49
- 42a. Length 35–40 mm.; segments as many as 90; blood red *F. magna* Friend
 b. Length not more than 25 mm.; segments as many as 65; blood colorless 43
- 43a. Maximum segments not more than 40; brain posteriorly emarginate
F. emarginata Bretscher
- b. Maximum segments more than 45; brain straight or convex posteriorly 44
- 44a. Peptonephridia weakly branched or with single posterior branch 45
 b. Peptonephridia with several well-developed branches at end 48
- 45a. Length up to 12 mm.; peptonephridia gutlike with a single branch at posterior end *F. auriculata* Bretscher
 b. Length up to 18–25 mm.; peptonephridia weakly branched at end 46
- 46a. Length up to 25 mm.; color greenish white or white *F. viridula* Issel
 b. Length not more than 20 mm.; color grayish white or white 47
- 47a. Nephridium with large anteseptal, nearly as large as postseptal; excretory duct leaves postseptal near its center *F. popoflana* Eisen
 b. Nephridium with large anteseptal, but only one-fourth to two-thirds as large as postseptal; duct leaves end of latter *F. leydigi* (Vejdovsky)
- 48a. Length 10–13 mm.; segments 45–50 *F. valdarnensis* Issel
 b. Length 20–25 mm. or more; segments 65 *F. agricola* Moore
- 49a. Fascicles with 4–6 (rarely 7) setae 50
 b. Fascicles with as many as 8 setae 60
- 50a. Segments 44; length 5.6 mm. (incompletely described from a single specimen)
F. omeri Stephenson
- b. Segments 50 or more; length 10 mm. or more 51
- 51a. Segments 50–55 52
 b. Segments 60 or more 54

- 52a. Brain concave anteriorly; spermathecal diverticula sessile upon ampullae
F. santaebarbarae Eisen
 b. Brain projects forward; spermathecal diverticula connected to ampullae by short stems 53
- 53a. Spermathecal duct shorter than ampulla and possessing several large ectal glands
F. humicola Bretscher
 b. Spermathecal duct about twice as long as ampulla and possessing a single large ectal gland *F. fruttensis* Bretscher
- 54a. Segments 70-80 *F. stephensoni* Moszynski
 b. Segments about 60-65 55
- 55a. Dorsal vessel arises anterior to XVII (see note under 56b, below) 56
 b. Dorsal vessel arises in XX-XXII 57
- 56a. Dorsal vessel arises in XV; excretory duct leaves the postseptal just behind the septum; peptonephridia with 4-6 tubules from a thick elongated base; color yellowish *F. santaerosae* Eisen
 b. Dorsal vessel arises in VII; excretory duct leaves the postseptal subterminally; peptonephridia with 2 sets of branches at posterior end; color whitish and glassy *F. perrieri* (Vejdovsky)
 [NOTE: Southern (1907) describes this species from Ireland with the dorsal vessel arising in XXII and the excretory duct leaving the postseptal just behind the septum.]
- 57a. Spermathecal diverticula reversed upon the ampulla; segments as many as 65; excretory duct leaves from the posterior third of the postseptal. . . . *F. reversa* Friend
 b. Diverticula not reversed; segments about 60; excretory duct leaves from the anterior third of the postseptal. 58
- 58a. Dorsal vessel arises in XXII; spermathecal diverticula long and directed forward *F. exserta* Bretscher
 b. Dorsal vessel arises in XVIII-XXI; spermathecal diverticula normal 59
- 59a. Sperm funnel 2-3 times as long as wide; anteseptal of nephridium nearly as large as postseptal *F. minuta* Bretscher
 b. Sperm funnel 5-6 times as long as wide; anteseptal of nephridium half as large as postseptal *F. cognetti* Bretscher
- 60a. Brain posteriorly concave; excretory duct leaves at terminus of postseptal; spermathecal duct with 2 large ectal glands *F. sardorum* Cognetti
 b. Brain posteriorly convex; excretory duct leaves the postseptal near the septum; no ectal glands 61
- 61a. Brain anteriorly convex; skin very glandular; peptonephridia with short base and 2 long, slender branches that may be bifurcated near their tips. *F. glandulosa* Southern
 b. Brain anteriorly concave; skin normal; peptonephridia with numerous small, short branches *F. polychaeta* Bretscher
- 62a. Spermathecae with 3 diverticula or pouches 63
 b. Spermathecae with 4 or more diverticula 65
- 63a. Segments 25-40; spermathecae with 2 lateral and 1 posterior enlargements or pouches *F. helvetica* Bretscher
 b. Segments about 60; diverticula normal pouches 64
- 64a. Length 12-17 mm.; setae as many as 4 per fascicle *F. aurita* Issel
 b. Length 18-20 mm.; setae as many as 7 per fascicle *F. ulmicola* Friend
- 65a. Setae as many as 16 per fascicle; parasitic on *Allolobophora robusta*
F. parasitica Cernosvitov
 b. Setae not more than 9 per fascicle; not parasitic 66
- 66a. Spermathecal diverticula numerous and small, 30 or more 67
 b. Spermathecal diverticula few in number, not more than 12 or so 68

- 67a. Setae 4-8 per fascicle; 30 or more small, sessile spermathecal diverticula in 2 rows around ampullae; peptonephridia much branched. *F. hegemon* (Vejdovsky)
 b. Setae not more than 6 per fascicle; spermathecal diverticula very numerous, clustering about intestine and ampulla (diagram shows 3 rows of sessile spheres about base of ampulla); peptonephridia small and little branched. *F. microcara* Friend
- 68a. Length 30-45 mm.; segments 90-95. *F. gigantea* Dequal
 b. Length not more than 25 mm.; segments not more than 90. 69
- 69a. Setae not more than 2 per fascicle. 70
 b. Setae 4 or more per fascicle. 72
- 70a. Spermathecae with 10-12 small, rounded (reduced) diverticula. *F. oligosetosa* Nusbaum
 b. Spermathecae with fewer and irregular diverticula. 71
- 71a. Length 3-4 mm.; segments 25-30; spermathecae with remarkable glandular diverticula, irregular in shape and leaving ampulla in irregular manner; no ectal gland; dorsal vessel arises in XIV. *F. nigrina* Friend
 b. Length about 10 mm.; segments about 40; spermathecae with small ampullae and with 2 different sizes of unequal diverticula; large ectal gland; dorsal vessel arises in XVII. *F. irregularis* Bretscher
- 72a. Setae as many as 4 per fascicle (rarely 5). 73
 b. Setae as many as 6 or more per fascicle. 81
- 73a. Spermathecae with long canal devoid of enlarged ampullae, bearing 4 small sessile lateral pouches. *F. quadriglobulata* Bretscher
 b. Spermathecae possess normal enlarged ampullae. 74
- 74a. Small worms, length 5-10 mm.; segments 35-40. 75
 b. Larger worms, exceeding 10 mm.; segments 50-69. 77
- 75a. Spermathecae with rough covering of cellular outgrowths (diverticula ?)
F. arborea Friend
 b. Spermathecae with sessile glands or diverticula. 76
- 76a. Length 5 mm.; peptonephridia with several definite branches. *F. humilis* Friend
 b. Length 10 mm.; peptonephridia long, twisted pouches, no branching observed
F. lacustris Bretscher
- 77a. Segments 50; spermatheca consists of long duct and spherical ampulla with a coronet of glands (diverticula ?). *F. coronata* Friend
 b. Segments 57-69; spermathecal ampullae with well-developed diverticula. 78
- 78a. Length 10-20 mm.; segments not more than 65; spermathecal ampullae possessing 3-6 diverticula. 79
 b. Length 25-30 mm.; segments more than 65; spermathecal ampullae possessing a whorl of 5-8 sessile diverticula at base. 80
- 79a. Length 8-15 mm.; segments 60-65; spermathecal ampullae with 3-6 finger-like diverticula; excretory duct arises from anterior half of postseptal
F. digitata Cognetti
 b. Length 10-20 mm.; segments 64; spermathecal ampullae flask-shaped with 4 evaginations at base; excretory duct from end of postseptal. *F. callosa* (Eisen)
- 80a. Prostomium blunt and rounded; brain concave anteriorly. *F. agilis* Smith
 b. Prostomium studded with little, rounded glandular knobs; brain normal (i.e., not mentioned as being otherwise). *F. longa* Moore
- 81a. Setae as many as 6 (occasionally 7) per fascicle. 82
 b. Setae as many as 8 per fascicle. 99
- 82a. Spermathecal ampullae with diverticula arranged in 2 rows of 4 large-stemmed vesicles; length 12-15 mm.; segments about 70. *F. bedoti* Bretscher
 b. Spermathecal diverticula in a single row at base of ampulla. 83
- 83a. Maximum length not more than 17 mm.; segments not more than 59. 84
 b. Maximum length 18 mm. or more; maximum segments 60 or more. 93

- 84a. Length 8-10 mm.; segments not more than 45. 85
 b. Length 10 mm.; segments 50-59. 86
 85a. Segments about 30; sperm funnel without collar or neck. (Incompletely described.)
F. clara Friend
 b. Segments up to 45; sperm funnel with noticeable collar. (See 94a, below.)
F. lobifera var. *minor* Friend
 86a. Spermathecal ampulla with about 10 diverticula. 87
 b. Spermathecal ampullae with not more than 7 diverticula. 88
 87a. Clitellum with glands in transverse rows with clear fields between; brain convex anteriorly; peptonephridia clearly branched; 2 ectal glands on spermathecal duct. *F. beddardi* Bretscher
 b. Clitellum without transverse rows of glands or clear fields between; brain concave anteriorly; peptonephridia large, tubular, unbranched, with tuberculate surface; no ectal glands. *F. douglasensis* Welch
 88a. Length 9-17 mm.; segments 52-59; brain anteriorly concave
F. tenera Smith and Welch
 b. Length not more than 15 mm.; segments not more than 55; brain anteriorly convex 89
 89a. Brain about square; peptonephridia branched and secondarily bifurcated. (Incomplete description from one specimen.) *F. terrestris* Bretscher
 b. Brain $1\frac{1}{2}$ to 2 times as long as broad; peptonephridia gutlike without branches or only weakly branched. 90
 90a. Spermathecal ampulla with as many as 4 diverticula and duct with ectal glands. . . 91
 b. Spermathecal ampullae with 5 or 6 diverticula; duct without ectal glands. 92
 91a. Length 10 mm.; spermathecal ampulla a gradual swelling of the duct; 4 spherical diverticula; 3 ectal glands; excretory duct terminal. *F. alpinula* Bretscher
 b. Length 12-15 mm.; spermathecal ampulla pyriform; 2-4 spherical diverticula; 1 large ectal gland; excretory duct leaves postseptal just behind septum
F. michaelsoni Bretscher
 92a. Head pore at tip of prostomium; peptonephridia unusually long, extending through 3 segments (III-V); sperm funnel of unusual shape, that is, saclike with narrow neck. *F. anglica* Friend
 b. Head pore and sperm funnel normal (by inference, since they are not mentioned in descriptions); peptonephridia normal, stout, with anterior processes and simple terminus. *F. hillmani* Friend
 93a. Maximum number of segments not more than 65. 94
 b. Maximum number of segments as many as 67 and 70. 98
 94a. Brain slightly concave posteriorly; spermathecae with ninepin-shaped ampullae with 6 massive, rounded glands like diverticula. *F. lobifera* Vejdovsky
 b. Brain posteriorly convex; spermathecal diverticula of usual pouchlike nature. . . . 95
 95a. Sperm funnel twice as long as broad; collar small and narrow. *F. udei* Bretscher
 b. Sperm funnel 3-4 times as long as broad; collar normal. 96
 96a. Excretory duct leaves postseptal subterminally; 3-5 stemmed, spherical diverticula at base of spermathecal ampulla. *F. galba* (Hoffmeister)
 b. Excretory duct arises near septum; spermathecal diverticula subdivided to give the appearance of 7 or more sessile spheres at base of ampulla. 97
 97a. Dorsal vessel arises in IX (?); no sperm sac described. *F. antarctica* Beddard
 b. Dorsal vessel arises in XXII; large sperm sac extends forward from the septum X/XI. *F. sacculata*, nov. sp.
 98a. Length 22 mm.; segments 70; brain ovoid; spermathecal ampulla with a row of 6-7 bladderlike diverticula. *F. californica* Eisen
 b. Length 24-33 mm.; segments 62-67; brain anteriorly concave, posteriorly convex; 3-4 sessile, unequal, lobular diverticula at base of ampulla. . . *F. firma* Smith and Welch

EXPLANATION OF PLATES

(All figures drawn with the aid of a camera lucida.)

PLATE 10

Fridericia sacculata

Fig. 1. A fascicle of setae from segment XLVIII. From life, $\times 360$.

Fig. 2. A ventral fascicle of setae from segment VI. From life, $\times 360$.

Fig. 3. The brain, dorsal aspect. From life, $\times 120$.

Fig. 4. Lateral view of anterior end of worm, showing the papillose skin and the position of the head pore. From life, $\times 72$.

Fig. 5. Dorsal view of the circulatory system in the anterior end. Four aortic arches present. From life, $\times 72$.

Fig. 6. Nephridium. From life, $\times 130$.

Fig. 7. Peptonephridium. From life, $\times 120$.

Fig. 8. Sperm funnel with sperm protruding. The funnel to the right is the same funnel contracted. The long, coiled sperm duct terminating in the penial bulb is shown in ventral aspect. From life, $\times 360$.

Fig. 9. Lateral view of segments IV-VII, showing spermathecae and septal glands. From life, $\times 49$.

Fig. 10. A chylus cell from prepared slide. Cilia upon surface of chylus and ental epithelial cells flattened. $\times 660$.

Fig. 11. Penis projected from living worm under the pressure of the cover glass. $\times 72$.

Fig. 12. Cross-section of ectal glands and spermathecal duct. $\times 660$.

Fig. 13. Nucleate and nonnucleate lymphocytes from the body cavity. $\times 360$.

Fig. 14. A section through the penial bulb from serial sections. $\times 360$.

Fig. 15. Dorsal view of segments IV-VII, showing the spermathecae and septal glands. From life, $\times 72$.

Fig. 16. Lateral aspect of segments IX-XIV, showing the sperm sac and also spermatozoa extruded through the dorsal pore in XI. From life, $\times 49$.

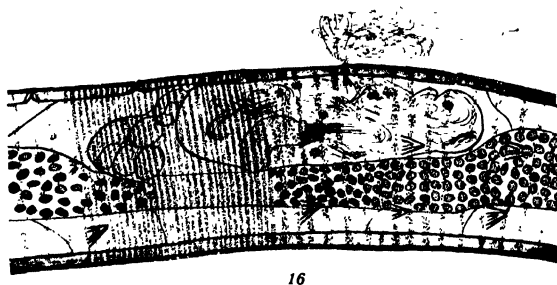
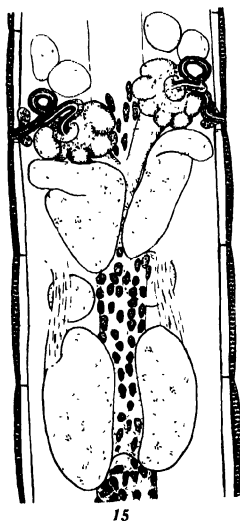
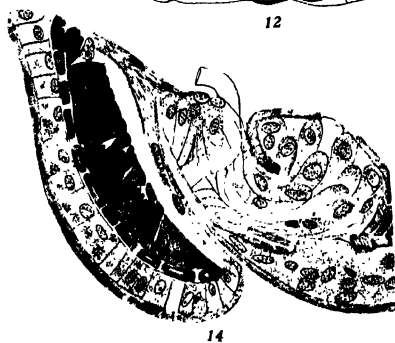
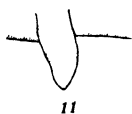
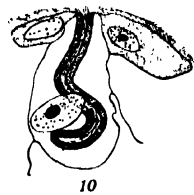
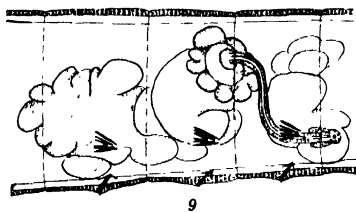
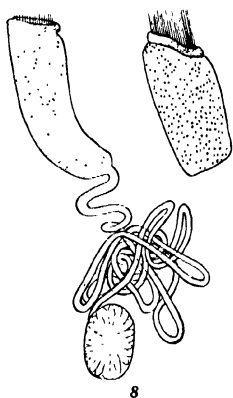
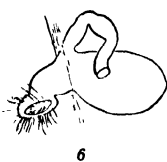
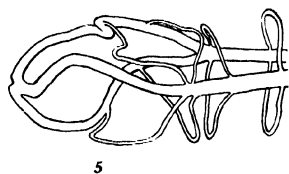
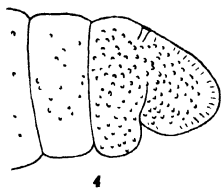
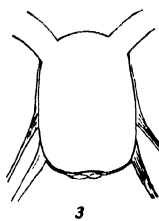
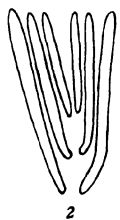
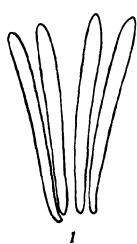


PLATE 11

Fridericia losangelensis

Fig. 17. Lateral aspect of the anterior end, with visible internal organs outlined in situ. From life, $\times 60$.

Fig. 18. Dorsal view of brain. From life, $\times 120$.

Fig. 19. Nephridium. From life, $\times 640$.

Fig. 20. Setae from somite XXII. $\times 640$.

Fig. 21. Chylus cell, a composite drawing from three consecutive sections of a prepared series. $\times 1184$.

Fig. 22. Dorsal aspect of spermathecae in living worm. $\times 60$.

Fig. 23. Nucleate and nonnucleate lymphocytes. From life, $\times 280$.

Fig. 24. A composite from a series of camera lucida drawings from the ectal pore to the communication of the spermathecae with the oesophagus. $\times 130$.

Fig. 25. A cross-section through the oesophagus and related structures in segment V, showing the communication of the spermathecae with the oesophagus. $\times 360$.

Fig. 26. Chylus cells from a prepared cross-section, showing the nature of the ental epithelial cells and the relation of the blood sinus to the intestinal mucosa. $\times 660$.

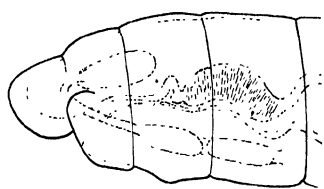
Fig. 27. Dorsal vessel with cardiac body within and chlorog cells without. $\times 660$.

Fig. 28. A sperm funnel with sperm protruding, drawn from a longitudinal series. $\times 240$.

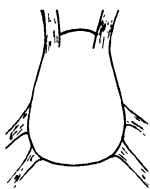
Fig. 29. A tangential section of skin of the clitellum. Glandular cells with clear spaces in between. $\times 640$.

Fig. 30. Peptonephridium from 2 consecutive sections from a longitudinal series. The dotted line indicates the single branch that passes dorsad. $\times 240$.

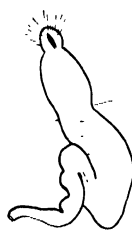
Fig. 31. Penial bulb from prepared sections. Cross-section. $\times 460$.



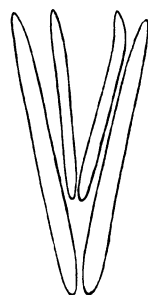
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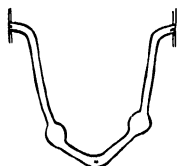
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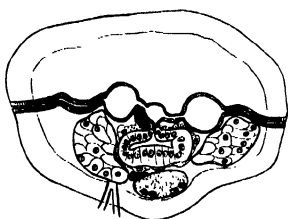
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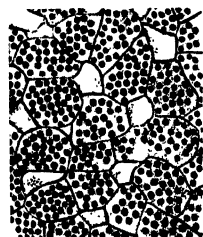
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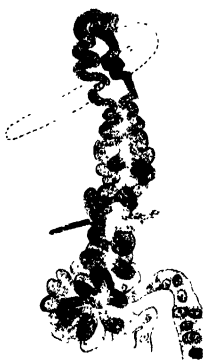
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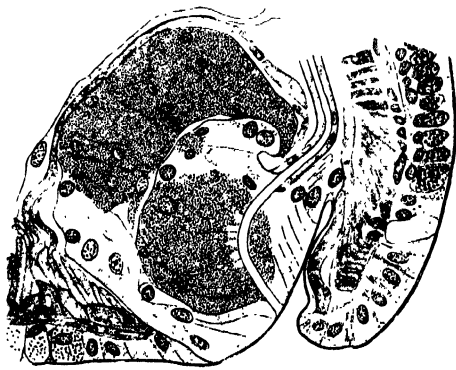
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**CALIFORNIA ISOPODS OF THE GENUS
PORCELLIO WITH DESCRIPTIONS OF A
NEW SPECIES AND A NEW SUBSPECIES**

BY

MILTON A. MILLER

UNIVERSITY OF CALIFORNIA PUBLICATIONS IN ZOÖLOGY

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CALIFORNIA ISOPODS OF THE GENUS PORCELLIO WITH DESCRIPTIONS OF A NEW SPECIES AND A NEW SUBSPECIES

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INTRODUCTION

NORTH AMERICA in general, California in particular, is a territory considered to be poor in species of terrestrial isopods in comparison with other regions, particularly Europe, notwithstanding the probability that a more thorough investigation of the first-named region will bring to light many hitherto undescribed species (Arcangeli, 1932*b*). Including the new forms here described, there are reported from California only five species of *Porcellio*, a genus with about three hundred species. This local paucity of species becomes even more apparent when it is noted that many, if not most, of our species are importations, and may indeed be only recently established in some localities, as is true of *Armadillidium vulgare* Latreille (Essig, 1926). The present paper adds a new species and a subspecies to an already overburdened genus, and also furnishes keys, descriptions, and figures which will make identification of the California species of *Porcellio* easier and at the same time more accurate.

SPECIES CHARACTERISTICS

Perhaps the greatest difficulty with which one has to deal in systematic work with this group is the inadequate description unaccompanied by critical figures, which in reality covers a group of related species. The unavailability of the type specimen increases this confusion of species. The prime requisite is to find critical diagnostic characters for use in systematic descriptions.

It is generally recognized that characters associated with the inherently stable reproductive system are less subject to variation and hence are excellent for diagnosis. The genus *Porcellio*, like most isopods, is sexually dimorphic with respect to the first two pairs of pleopods, which in the male are developed into a copulatory organ. The exopodites, or opercular plates, of these two pairs of abdominal appendages show easily recognized specific differences (figs. 14-18, 24-29). Another sexually dimorphic structure in the male, the ischiopodite (the second free joint from the body) of the seventh pair of walking legs, also exhibits characteristic differences (figs. 3, 5, 6, etc.). The foregoing characters have been suggested and extensively used by recent European authors, particularly Arcangeli (1932*a*), in differentiating species of *Porcellio* and of other genera of terrestrial isopods. In addition to, and in conjunction with, these characters, other more accessible ones, not limited to one sex, are also delineated.

KEY TO THE CALIFORNIA SPECIES OF PORCELLIO

1. Surface of body smooth or minutely granular. 2
- 1'. Surface of body roughly granulate or tuberculate. *Porcellio scaber* Latreille
and *P. s. americanus* Arcangeli.
2. Flagellum of second pair of antennae with first article less than one-half length of second. *Porcellio littorina*, sp. nov.
- 2'. First article of flagellum not less than half the length of second. 3
3. Surface of body minutely granular. Telson produced in a spatulate process. *Porcellio spinicornis occidentalis*, subsp. nov.
- 3'. Surface of body smooth. Telson triangular or produced in a triangulate process. 4
4. Articles of flagellum subequal. Exopodite of first pair of pleopods of male rounded at tip. *Porcellio formosus* Stuxberg.
- 4'. First article of flagellum longer than second. Exopodite of first pair of pleopods in male acuminate at tip. *Porcellio laevis* Latreille.

The following descriptions give only the salient features useful in identification.

Porcellio scaber Latreille

(figs. 2, 6, 10, 17, 24)

Body twice or a little less than twice as long as wide. Surface of body roughly granular. Color usually a uniform dark gray, black, or brown, often with a lighter marginal border.

Head with median lobe triangular, apex obtuse; the anterolateral lobes rounded and extending as far as the median lobe. Flagellum of second pair of antennae composed of two subequal articles or with first slightly shorter than second.

Segments of thorax subequal. Ischiopodite of seventh pair of walking legs in the male with a triangular process, the margin opposite which is slightly incurved.

Abdomen with telson produced in a triangulate process. Exopodite of first pair of pleopods in male with a humplike process on its outer distal margin. Inner distal margin furnished with spines.

Localities.—Taken only at Moss Beach, San Mateo County, California, at mouth of small creek.

Porcellio scaber americanus Arcangeli (1932b)

(figs. 1, 5, 11, 18)

This subspecies differs from *P. scaber* as follows:

1. Surface granulations more pronounced.
2. Pigmentation greater, especially in males, and extends to ventral side of body, not even excluding the pleopods in females.

Fig. 1. *Porcellio scaber americanus*, exopodite of right pleopod of first pair, female, ventral aspect. $\times 25$.

Fig. 2. *P. scaber*, exopodite of right pleopod of first pair, female, ventral aspect. $\times 25$.

Fig. 3. *P. littorina*, right seventh leg of male. $\times 25$.

Fig. 4. *P. laevis*, flagellum of second antenna. $\times 12$.

Fig. 5. *P. scaber americanus*, ischiopodite of seventh leg of male. $\times 25$.

Fig. 6. *P. scaber*, ischiopodite of seventh leg of male. $\times 25$.

Fig. 7. *P. littorina*, head and first thoracic segment, male (left antenna removed). $\times 12$.

Fig. 8. *P. spinicornis occidentalis*, head and first thoracic segment, male (left antenna removed). $\times 12$.

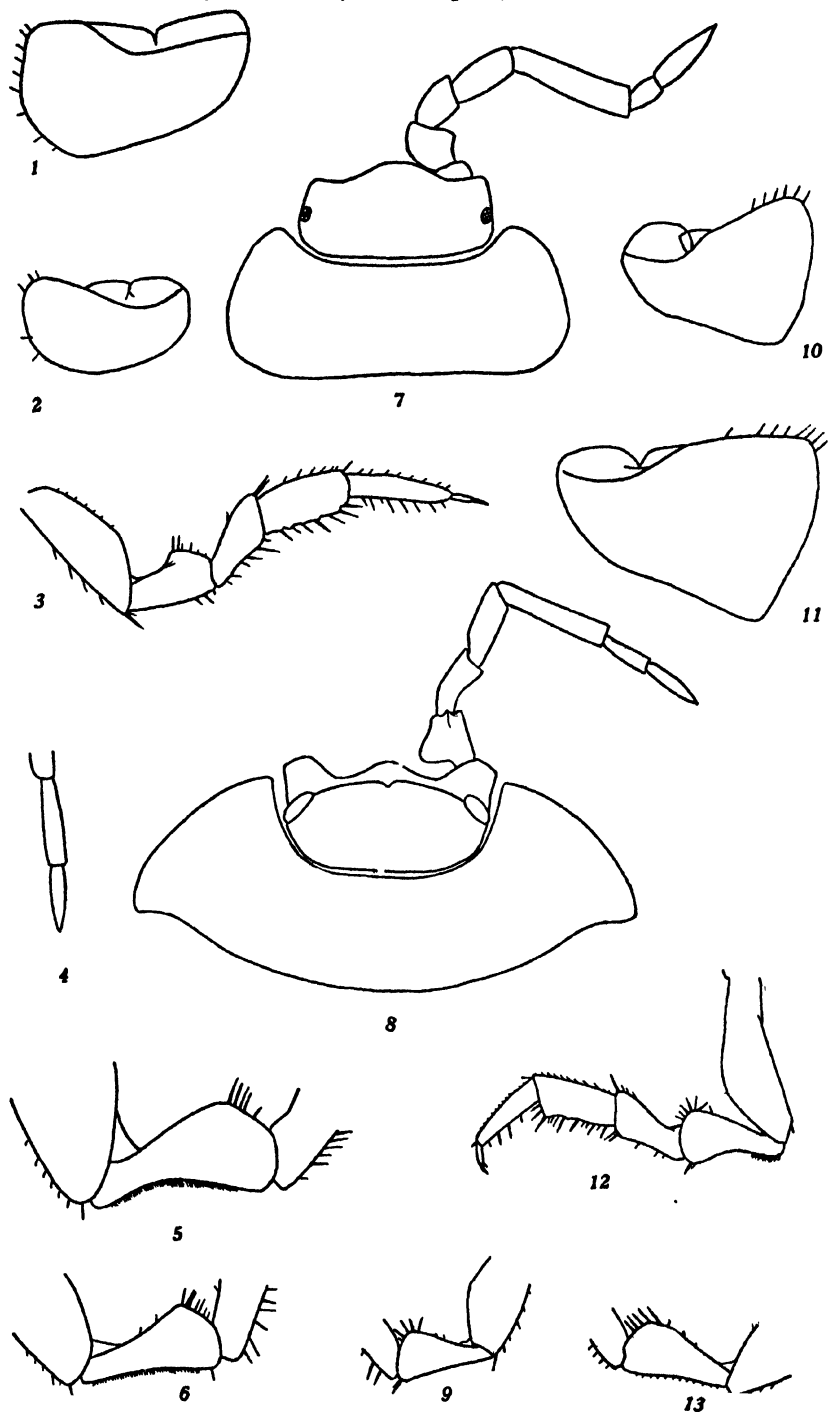
Fig. 9. *P. laevis*, ischiopodite of seventh leg of male. $\times 12$.

Fig. 10. *P. scaber*, exopodite of left pleopod of second pair, female, ventral aspect. $\times 25$.

Fig. 11. *P. scaber americanus*, exopodite of left pleopod of second pair, female, ventral aspect. $\times 25$.

Fig. 12. *P. spinicornis occidentalis*, left seventh leg of male. $\times 12$.

Fig. 13. *P. spinicornis*, ischiopodite of seventh leg of male (after Richardson, 1905).



Figures 1-13
(All figures drawn with aid of camera lucida)

3. Frontal lobe of head more acute.

4. Lower margin of ischiopodite of seventh leg in male more distinctly bowed.

5. Exopodites of first two pairs of pleopods in both male and female exhibit slight differences.

Localities.—Described from San Mateo, California, and British Columbia. Commonly found all around San Francisco Bay, and at Carmel, California. Limited to damp situations such as the banks of small creeks or streams, and gardens.

Porcellio littorina, sp. nov.

(figs. 3, 7, 14, 15, 19)

Body smooth, nearly two and a half times as long as wide, 6.0 mm.: 2.5 mm. Color brown, with a band of irregular light spots on either side of median dorsal line. Lateral margins of thorax somewhat lighter.

Head but slightly immersed in first thoracic segment. Front produced in three lobes, anterolateral lobes not prominent, almost vertical; median lobe large and rounded. Eyes small, composed of about six to eight ocelli, situated at base of anterolateral lobes. First pair of antennae rudimentary. Second pair of antennae extend to posterior margin of second thoracic segment. First article of flagellum short, one-third the length of second.

Segments of thorax subequal. First segment with anterolateral angles only slightly produced around head. Posterior margins of first three thoracic segments straight, rounded at lateral angles. Posterolateral angles of remaining thoracic segments become progressively more and more produced posteriorly, but not extremely so. Legs slightly spiny. Ischiopodite of seventh walking legs in male with a pronounced, humplike process.

Abdomen as usual in the genus, with lateral parts of first and second segments covered by last thoracic segment. Posterolateral parts of third, fourth, and fifth segments acuminate produced almost at right angles to transverse axis. Telson triangular, only slightly produced. Exopodite of first pleopods in male bears a distinctive knoblike process on its outer distal margin.

Localities.—Twelve specimens, including one female with embryos in marsupium, collected under rocks above high tide at Bay Farm Island, Alameda, California, along with two species of *Actoniscus*.

This species is apparently closely related to *P. parvicornis* Richardson, from the Bermudas.

Fig. 14. *P. littorina*, exopodite of left pleopod of first pair, male, ventral aspect. $\times 55$.

Fig. 15. *P. littorina*, exopodite of left pleopod of second pair, male, ventral aspect. $\times 55$.

Fig. 16. *P. laevis*, exopodite of left pleopod of first pair, male, ventral aspect. $\times 25$.

Fig. 17. *P. scaber*, exopodite of right pleopod of first pair, male, ventral aspect. $\times 25$.

Fig. 18. *P. scaber americanus*, exopodite of right pleopod of first pair, male, ventral aspect. $\times 25$.

Figs. 19–24. Fifth abdominal segment, telson, and uropoda. $\times 12$.

19. *P. littorina*.

20. *P. spinicornis occidentalis*.

21. *P. spinicornis* (after Richardson, 1905).

22. *P. laevis*.

23. *P. formosus* (after Arcangeli, 1932b).

24. *P. scaber*.

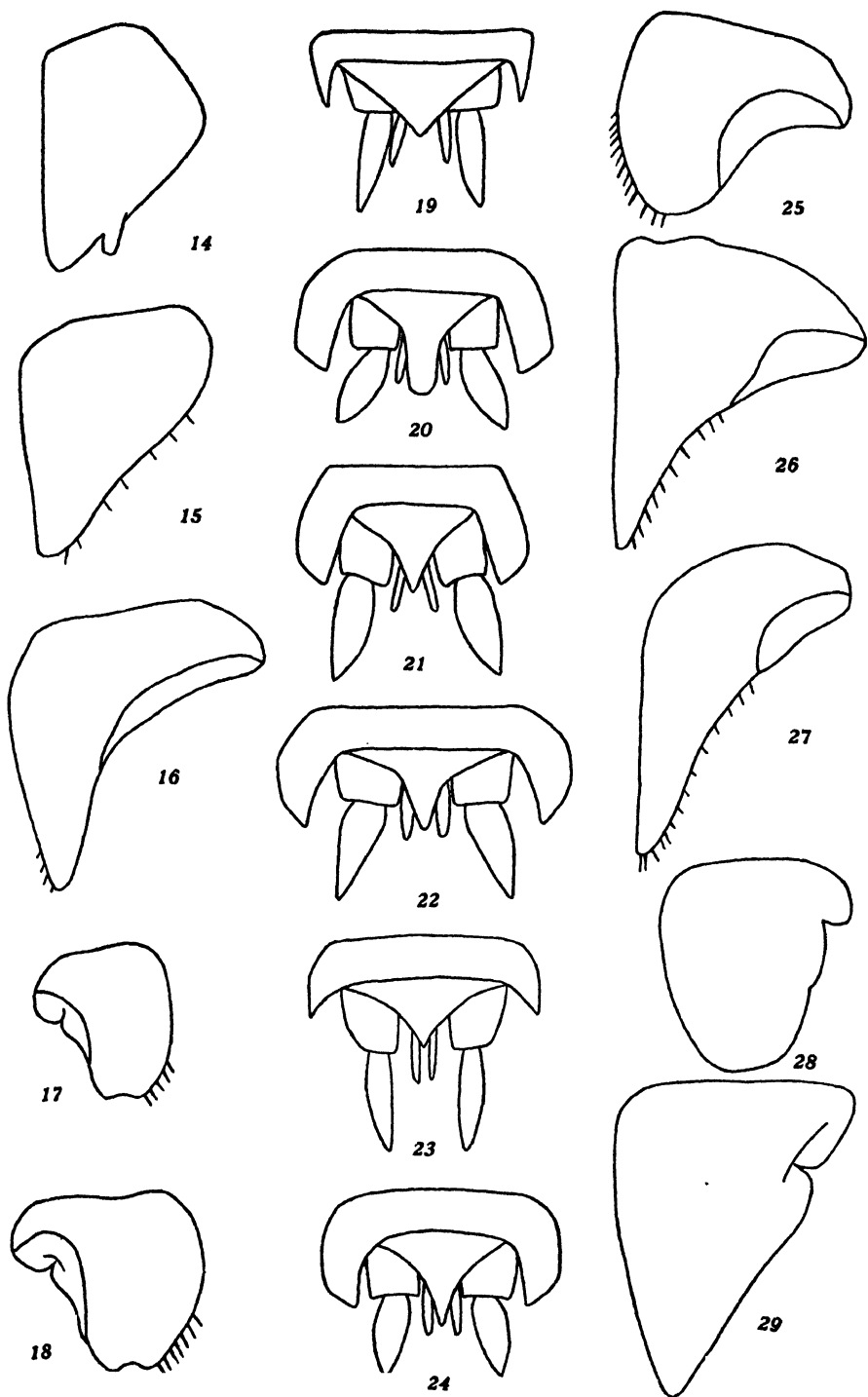
Fig. 25. *P. spinicornis occidentalis*, exopodite of left pleopod of first pair, male, ventral aspect. $\times 25$.

Fig. 26. *P. spinicornis occidentalis*, exopodite of left pleopod of second pair, male, ventral aspect. $\times 25$.

Fig. 27. *P. laevis*, exopodite of left pleopod of second pair, male, ventral aspect. $\times 25$.

Fig. 28. *P. formosus*, exopodite of left pleopod of first pair, male, ventral aspect (after Arcangeli, 1932b).

Fig. 29. *P. formosus*, exopodite of left pleopod of second pair, male, ventral aspect (after Arcangeli, 1932b).



Figures 14-29
(All figures drawn with aid of camera lucida.)

Porcellio spinicornis Say *occidentalis*, subsp. nov.

(figs. 8, 12, 20, 25, 26)

Body oval, depressed, with surface minutely granular, about one and three-fourths as long as wide, 12 mm.: 7 mm. Color dark grayish brown, with a broad band of paler spots on either side of median line, and lateral to these bands a single longitudinal line of larger light spots.

Head deeply immersed in first thoracic segment. Front of head produced in three lobes, the anterolateral lobes slightly more produced than the median lobe, which is rounded. Eyes small, oval, and situated at base of anterolateral lobes. Articles of flagellum of second pair of antennae subequal.

Segments of thorax subequal. Anterolateral parts of first thoracic segment strongly produced to surround head, extending almost to tip of anterolateral lobes. Posterolateral angles of all thoracic segments posteriorly produced. Legs spiny. Ischiopodite of seventh pair of legs in male with a process, the margin opposite which is strongly incurved.

Abdomen as usual in the genus, with lateral parts of first two segments concealed by last thoracic segment, and those of the third, fourth, and fifth produced to continue the oval outline of body. Telson produced at apex in a spatulate process with a dorsal shallow groove extending to tip of process. Telson extends to tip of endopodites of uropods. Exopodite of first pleopods in male broadly rounded at apex with outer margin entire and incurved.

The description given above reveals that the new subspecies differs from *P. spinicornis* in the following respects:

1. Articles of flagellum subequal; not the first longer than the second.
2. Median lobe of head not so expanded.
3. Ischiopodite of seventh legs of male more bowed (a difference comparable to that between *P. scaber* and *P. s. americanus*).
4. Telson spatulate rather than triangular.

Species have been separated on smaller differences than these here given subspecific value. The similarity of the two forms, *P. spinicornis* and *P. s. occidentalis*, especially in the first two pairs of pleopods, does not allow specific separation. However, the distribution of the two forms, *P. spinicornis* reported from northeastern United States and northern Europe, and *P. s. occidentalis* from the Pacific Coast, coupled with the structural differences, does warrant subspecific designation.

Localities.—Common in the San Francisco Bay region and along the coast of California; I have also found it in moist situations at Davis, California; very commonly associated with *P. laevis* Latreille, from which it can easily be distinguished by its granular surface, its spatulate telson, and its greater propensity for feigning death.

Porcellio formosus Stuxberg (1875)

(figs. 23, 28, 29)

Several authors, including Budde-Lund (1885), have suggested the possibility that *P. formosus* might be *P. laevis*. Doubt of the existence of this species was based largely on the fact that specimens of it had not as yet been found in its type localities (San Francisco and San Pedro), although reported by Stuxberg himself as "frequentissimus." However, the species is now definitely established by Arcangeli's publication (1932b) of supplementary description and figures (the first, to my knowledge) of specimens of *P. formosus* collected

in Louisiana and Texas. Whether or not the species will again be found in California remains to be seen, but since it was first reported from California and now seems to be a valid species, it is desirable to supply a few characters separating it from *P. laevis*, with which it is most likely to be confused, and to reproduce in outline some of Arcangeli's figures.

There are a number of differences between *P. formosus* and *P. laevis* (see description below). In the former, the lobes of the head are not so pronounced; the articles of the flagellum are subequal, not the first longer than the second; the telson is relatively wider, the apex not so produced. But by far the most easily recognized and clear-cut distinctions between the two species are to be found in the first two pairs of pleopods, especially in the exopodite of the first pair of pleopods in the male. In *P. formosus* this exopodite is distally rounded and indented on its proximal outer margin, whereas in *P. laevis* it is acuminate, with its lateral margin entire.

Porcellio laevis Latreille

(figs. 4, 9, 16, 22, 27)

Body smooth and shiny, twice as long as wide, 14 mm.: 7 mm. Color dark brown, with a longitudinal band of a lighter color in wavy stripes on either side of median line.

Head with front produced in three lobes, the lateral lobes rounded and larger than the median, which is triangulate. Second pair of antennae with flagellum of two unequal articles, the first longer than the second, although in some specimens the articles are subequal.

Segments of thorax subequal, with anterolateral parts of first segment produced to surround lateral parts of head. Posterior margins of first three segments practically straight. Posterolateral angles of remaining segments progressively more and more produced posteriorly. Distal parts of legs spiny. Ischiopodite of seventh leg of male with triangulate process, the margin opposite which is straight.

Abdomen as usual in the genus. Apex of telson produced in a triangulate process with a groove extending the length of it. Both rami of uropods extend beyond tip of telson. Exopodites of first and second pairs of pleopods in male similar, acuminate at apex.

Localities.—The commonest species of the genus encountered in the San Francisco Bay region. In California it is reported from Berkeley, Oakland, Alameda, Moss Beach, Davis, San Diego, Monterey Bay, Raymond, and Colfax.

SUMMARY

Keys, descriptions, figures, and localities are given for all known species of *Porcellio* in California, including descriptions of a new species, *P. littorina*, and a new subspecies, *P. spinicornis occidentalis*, prefaced by a brief discussion of species characters.

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A REVIEW OF THE GENERA
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HEMICYCLOPS BOECK
(COPEPODA, CYCLOPOIDA)

WITH THE DESCRIPTION OF A NEW SPECIES
FROM THE NORTHEAST PACIFIC

BY

S. F. LIGHT AND OLGA HARTMAN

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DESCRIPTION OF A NEW SPECIES FROM THE
NORTHEAST PACIFIC

BY

S. F. LIGHT AND OLGA HARTMAN

THE GENUS *Clausidium* has been recorded from only two localities on the Pacific coast, one on Puget Sound, the other in southern California. A striking red copepod, clearly of this genus, has long been known to us to occur in considerable numbers on the body surfaces of the California ghost shrimp, *Callianassa californiensis* Dana, which abounds in the sandy mud flats of San Francisco, Bolinas, Tomales, and Bodega bays in central California. Since the species found in central California differs from the descriptions of the two species already described from the Pacific coast, an investigation was begun by Miss Janet Mabry at the suggestion of the senior author, under the impression that the form from central California was a third species. As will be seen below, this is not the fact, and since Miss Mabry found it necessary to drop the investigation in its early stages, it was continued by us and expanded into a review of *Clausidium* and the related, but less modified, genus *Hemicyclops* Boeck.

The two species of *Clausidium* that have been described from the Pacific are *C. vancouverense* (Haddon) from Nanaimo, British Columbia, and *C. californiense* Wilson from Newport Bay, southern California. A curious discrepancy existed with respect to the host species of *C. vancouverense* (Haddon), which was given as "*Callianassa pugettensis*" (1912, p. 86). Whether this should have read *Callianassa californiensis* Dana or *Upogebia pugettensis* (Dana) is doubtful; both hosts range widely along the shores of the northeast Pacific, the former from Alaska south to San Diego, California, the latter from Alaska to Lower California, and both harbor *Clausidium*.

Clausidium from central California does not agree with descriptions and figures of either Haddon or Wilson. Through the courtesy of the United States National Museum it has been possible to study and dissect one of the syntypes of *Clausidium californiense* (U.S.N.M. 64061) from Newport Bay. Furthermore, there were kindly put at our disposal collections made by Dr. G. E. MacGinitie and by Mr. Arthur B. Burch from Newport Bay (type locality of *C. californiense*), some by Miss Barbara Blanchard from Puget Sound, Washington, and others by Dr. Edith Berkeley from Nanaimo, British Columbia (type locality of *C. vancouverense*). In addition, numerous collections were available from San Francisco, Bolinas, and Tomales bays, in central California.

Careful comparisons of these materials have led to the conclusion that a single species of *Clausidium*, *C. vancouverense* (Haddon), ranges throughout the areas studied, and that the hosts of this species of *Clausidium* are the species of the genus *Callianassa*, and *Upogebia pugettensis*.

A revised list of species of *Clausidium* Kossmann, including known distributional records and references, follows:

1. *C. apodiforme* (Philippi) Adriatic and Mediterranean seas
Hersilia apodiformis Philippi, 1839; Milne Edwards, 1840; Heller, 1866; Claus, 1875; Canu, 1888; Embleton, 1903.
Clausidium testudo Kossmann, 1874 (*vide* Claus, 1875).
 Host: *Callianassa subterranea* Montagu.
2. *C. caudatum* (Say) St. John's River, Florida
Binoculus caudatus Say, 1818.
Clausidium caudatum, Wilson, 1922.
 Host: *Callianassa major* Say.
3. *C. vancouverense* (Haddon)
 Northeast Pacific from British Columbia south to Newport Bay, California
Hersilia (Clausidium) vancouverensis Haddon, 1912.
C. vancouverense, Wilson, 1922.
C. californiense Wilson, 1935.
 Hosts: *Callianassa californiensis* Dana.
Callianassa gigas Dana.
Upogebia pugettensis (Dana).
4. *C. dissimile* Wilson, 1922; 1932 Long Island Sound; Vineyard Sound
 Host: *Callianassa stimpsoni* Smith.

DESCRIPTIONS OF SPECIES

Clausidium vancouverense (Haddon)

(Figs. 1-11; plate 12, figs. 1, 2)

Measurements in millimeters of typical individuals from various localities:

	Female	Male
1. Nanaïmo, British Columbia		
Length with caudal rami.	1.40	0.76
Length of dorsal shield	1.0	0.40
Greatest width	0.74	0.38
2. Puget Sound, Washington		
Length with caudal rami.	1.62	0.8
Length of dorsal shield	1.26	0.54
Greatest width	0.94	0.4

EXPLANATION OF FIGURES 1-9

(All figures were drawn with the aid of a camera lucida)

Figs. 1-7. *Clausidium vancouverense* (Haddon)

Fig. 1. Female in ventral view, showing general arrangement of parts and gross structure ($\times 53$).

Fig. 2. Male in dorsal view. Antennae omitted ($\times 53$).

Fig. 3. Left second antenna, from female ($\times 83$).

Fig. 4. Right fifth leg of female ($\times 83$).

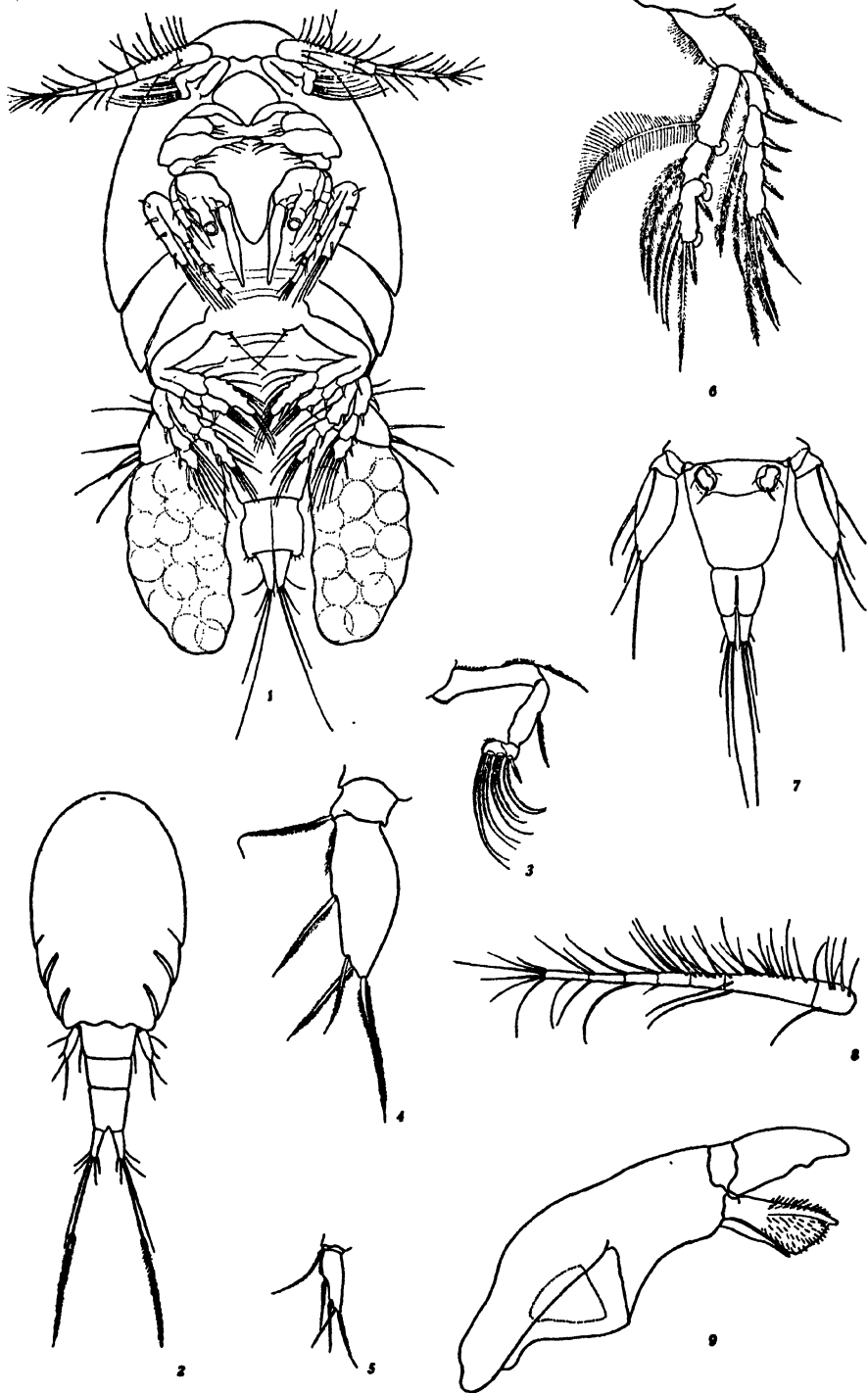
Fig. 5. Right fifth leg of male ($\times 83$).

Fig. 6. Right second leg of female ($\times 83$).

Fig. 7. Abdomen and genital segment in dorsal view ($\times 53$).

Fig. 8. First antenna of female ($\times 83$).

Fig. 9. Right mandible in posterior view ($\times 370$).



Figs. 1-9.

3. San Francisco Bay, northern California

Length with caudal rami.....	1.46	0.8
Length of dorsal shield.....	1.20	0.6
Greatest width	0.82	0.38

4. Corona del Mar, Newport Bay, California

Length with caudal rami.....	1.3-1.35	0.72
Length of dorsal shield.....	1.0	0.52
Greatest width	0.66	0.36

Female (measurements based on individuals from San Francisco Bay).—Cephalothorax a broad plate projecting posteriorly at sides (fig. 1), wider than long, greatest length (measured between anterior end of cephalothorax and ends of lateral plates) 0.6 mm.; length in median dorsal line 0.48 mm.; greatest width 0.66 mm. Second and third segments each 0.14 mm. long. Fourth and fifth thoracic segments covered by a dorsal plate 0.32 mm. long, its posterior edge free, strongly rounded in its median region, and terminating laterally in expanded wings.

Urosome (including genital segment and caudal rami) (fig. 7) about twice as long as wide, or slightly longer. Genital segment longer than wide, narrower than fifth thoracic segment. Genital openings dorsoectal, at anterior border of genital segment. Genital apertures with a sharp spine at posterior margin and 2 slender setae, one at either side. Second urosomal segment more or less trapezoidal, as wide as long, its dorsolateral borders thrown into chitinous folds about which the maxillipeds of male are fastened. Caudal rami trapezoidal in outline, tapering posteriorly and bearing distally 2 long plumose setae, a shorter, outer ectal seta, a minute seta on the inner ectal margin, and another inserted on the lateral margin, about one-third the distance from end of ramus (fig. 7).

First antennae 7-segmented; second segment the longest (fig. 8); segments 3 to 6 subequal; first antennae strongly setose. Second antennae 4-segmented; first segment the longest, with a plumose seta at its distal end and proximally with a row of stout spines and distally with short hairs (fig. 3); second segment with a plumose seta; third and fourth segments more or less fused; third segment with 2 slender setae and a short, stout, proximal one; fourth segment with 2 groups of long setae, seven in all (fig. 3).

Mandibles almost covered by a flaplike upper lip, their long, gently curved outer margins terminating distally in an articulating hook bearing a small knob on its inner side (fig. 9) and a row of slender setae near the cutting edge on its anterior side. Inner side of mandible terminating distally in an articulating piece which ends in a tooth, partly covered by short setae (fig. 9), with a slender lamella articulating near the ectal border (fig. 9).

EXPLANATION OF FIGURES 10-20

(All figures were drawn with the aid of a camera lucida)

Fig. 10. Left first leg of female in ventral view ($\times 233$).

Fig. 11. Left first leg of female, showing segments 2 and 3 of endopodite, seen from side facing median line ($\times 370$).

Figs. 12-20. *Hemicyclops pugettensis*

Fig. 12. Fifth thoracic segment and urosome of female in ventral view ($\times 53$).

Fig. 13. End of caudal ramus of female, showing length of setae ($\times 53$).

Fig. 14. Urosome of male in dorsal view ($\times 53$).

Fig. 15. Right first antenna of female ($\times 83$).

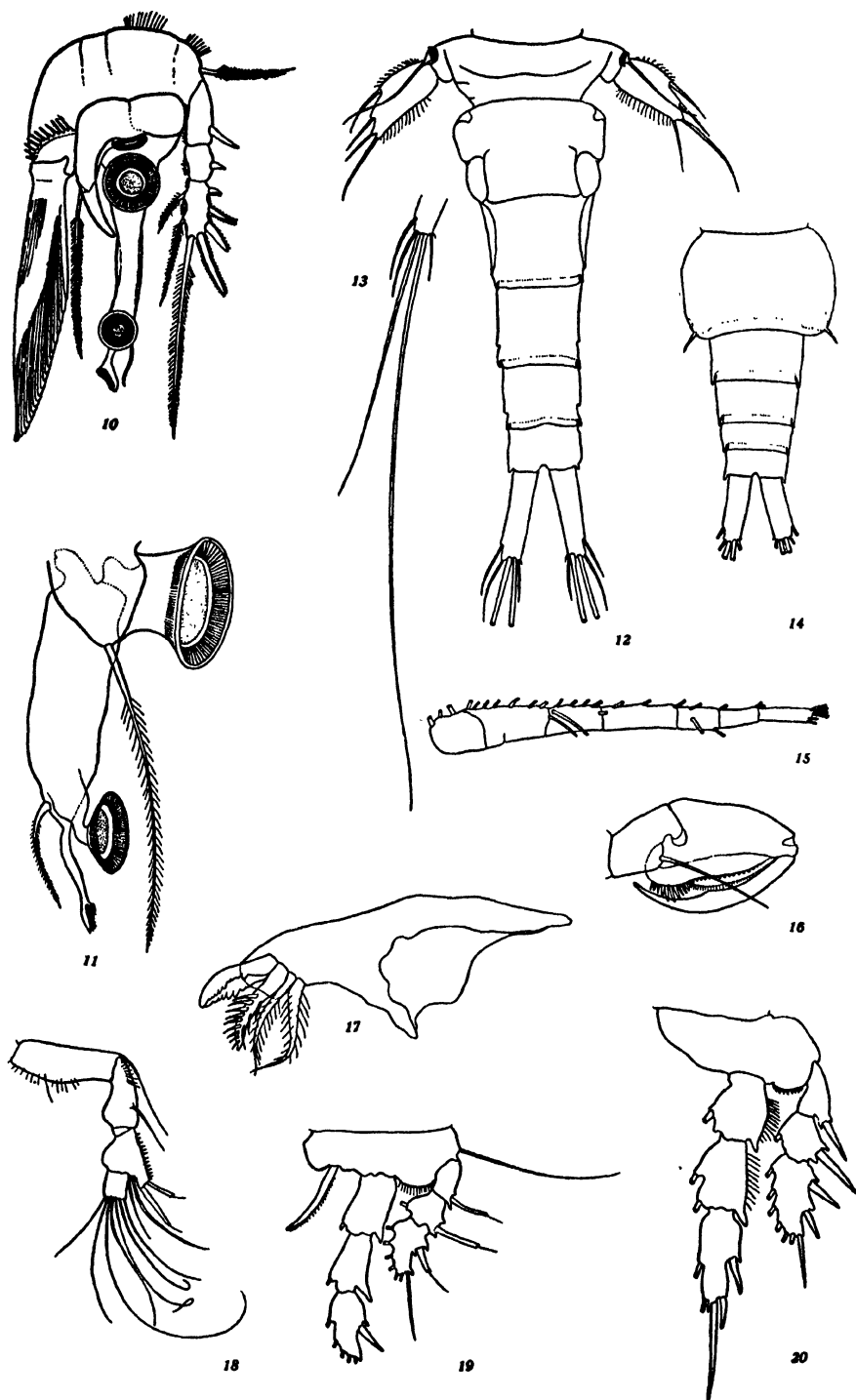
Fig. 16. Maxilliped of male ($\times 83$).

Fig. 17. Left mandible of female, palp removed ($\times 233$).

Fig. 18. Left second antenna of female ($\times 83$).

Fig. 19. Left leg of male ($\times 83$).

Fig. 20. Left fourth leg of female ($\times 83$).



Figs. 10-20.

First maxilla with 6 terminal setae in 2 groups of 3 each and 1 lateral seta on outer side. Second maxilla 2-segmented, proximal segment with 3 setae, distal segment with 3 spines, the distal spine being the stoutest.

Maxilliped 3-segmented, basal segment longest, distal segment hardly longer than broad; first and second segments each with 2 spines inserted near their middle; distal segment with 3 setae and 2 stout spines.

First pair of legs greatly modified (fig. 10); inserted at sides of flaplike lower lip (fig. 1); basal setae greatly enlarged to form stout spines which lie at sides of lower lip. Exopodite of 3 segments, its basal and second segments each with a spine; third segment with 4 outer spines and 3 inner setae (fig. 10). Endopodite of 3 segments, its basal segment a stout curved spine with a sucking disk near its base (fig. 10); second segment reduced, with only a long plumose seta (fig. 11); third segment terminating in a boot-shaped piece and having on its ventral side 2 conspicuous sucking disks (figs. 10, 11).

Second to fourth pairs of legs less modified, resembling one another (fig. 6); exopodites and endopodites 3-segmented. Setal formulae as follows (sp = spine, se = seta, su = sucking disk): Leg 2, exopodite: sp-0; sp-se; 3sp-5se; endopodite: su-se; 0-2se; 2su + 2sp-4se. Leg 3, exopodite: sp-0; sp-se; 3sp-5se; endopodite: su-se; 0-2se; 2su + 2sp-4se. Leg 4, exopodite: sp-0; sp-se; 3sp-5se; endopodite: su-se; 0-se; 2su + 2sp-3se. Fifth thoracic legs 2-segmented, basal segment with 1 seta, second segment with 4 lateral setae (figs. 4, 7). Short, minute hairs, more or less reduced, present along outer margins of distal segment.

Male (measurements based on individuals from San Francisco Bay).—Length without furcal setae 0.81 mm. or less; length with furcal setae 1.25 mm. or less; greatest width 0.36 mm. Differs from female chiefly in being smaller (pl. 12, figs. 1, 2), in having a more attenuate body form (fig. 2); caudal margin of dorsal shield with median emargination (fig. 2). Maxillipeds greatly modified for clasping second abdominal segment of female. Base of first thoracic leg with a plumose seta instead of stout spine as in female. Genital segment with a pair of long setae, their length slightly exceeding that of the segment which bears them.

Discussion.—The description of *C. vancouverense* as given by Haddon (1912) does not differ from the one given above except for the statement "mandibles bearing a tooth with smooth edges." The subterminal knob (fig. 9) called a tooth by Haddon is present, but it is easily overlooked unless the mandible lies in a favorable position. Haddon's figure (pl. 2, fig. 1, 1912) showing the relative position of the mouth parts, the thoracic legs, and the dorsal plates seems to be inaccurate in that the mouth parts are too much reduced and the spaces between these parts exaggerated. Also, the abdomen (unsegmented in Haddon's figure) shows segmentation clearly in all individuals examined.

The description given for *C. californiense* differs widely from the diagnosis of *C. vancouverense* here given, and had it not been for the study of syntype no. 64061, U. S. Nat. Mus., there would have been no hesitation in considering *C. californiense* a different species. In the syntype the abdomen consists of 3 segments, not a single segment; the antenna is 4-segmented, not 3-segmented; the distribution of the setae on its segments is as described above, the first leg is modified so that its exopod, not endopod, is simple and 3-segmented, the first and second segments have each a spine, the third has 4 spines and 3 setae. The endopod is greatly modified; the long, flattened spine is homologous with the stout setae on the basal segments of the other thoracic legs and is not a part of the ramus; the first endopodal segment is provided with a short curved spine, the second segment has a long plumose seta, the third segment is boot-

shaped. Setae and spines of other thoracic legs are as described above. Since syntype no. 64061 and other specimens from southern California agree in all details with individuals from other parts of the northeast Pacific except for their smaller size (cf. measurements given above), *C. californiense* is considered synonymous with *C. vancouverense*.

Hemicyclops Boeck, 1873

[Including *Platycheiron* Scott (*vide* Sars, 1913);

Lichomolgus (pro parte) (*vide* Sars, 1913); non *Hemicyclops* Claus, 1893.]

Type: *H. purpureus* Boeck, 1873.

Generic diagnosis.—Body cyclopoid; metasome and urosome clearly marked; metasome a broad shield, urosome slender. First antenna simple, of 7 segments, second and fourth segments exceeding others in length; second antenna 4-segmented. Mandibles with palpi. Swimming legs 1 to 4 normal, biramous, endopod and exopod each 3-segmented, the endopod exceeding exopod in length. Fifth leg of 2 segments, a short basal segment with 1 seta and a broad lamellar distal segment with 4 setae. Male resembles female, but smaller; maxilliped modified for clasping abdomen of female.

Discussion.—The affinities of *Hemicyclops* and *Clausidium* have already been discussed (*vide* Sars, 1913; Wilson, 1932). It is of interest that of the six species recognized, only the two reported from the Pacific have been associated with a host species. There is now to be added a third, *H. pugettensis* sp. nov. associated with *Callianassa gigas* Dana, along with *Clausidium vancouverense*.

We wish to express appreciation to Miss Barbara Blanchard for the collections used; also to Dr. G. E. MacGinitie for topotypes of *H. thysanotus* from the surface of *Hermisenda crassicornis*, from Corona del Mar, California.

A revised list of species of *Hemicyclops*, including known distributional records and references, follows:

1. *H. purpureus* Boeck, 1873.....Norway, Scotland
Sars, 1913; Wilson, 1932.
Lichomolgus littoralis Scott, 1892 (*vide* Sars, 1913).
2. *H. aberdonensis* (Scott and Scott).....Scotland, in bottom tow
Lichomolgus aberdonensis Scott and Scott, 1892.
H. aberdonensis, Sars, 1913.
3. *H. adherens* (Williams).....Wickford, Rhode Island; under stones, intertidal
Lichomolgus adherens Williams, 1907.
H. adherens, Wilson, 1932.
4. *H. americanus* Wilson, 1932.....Chesapeake Bay; bottom tow, in brackish water
5. *H. callianassae* Wilson, 1935
Corona del Mar, California; in gill chamber of *Callianassa californiensis* Dana
6. *H. thysanotus* Wilson, 1935
Elkhorn Slough, central California; on *Hermisenda crassicornis*
7. *H. pugettensis* sp. nov.
False Narrows, Puget Sound, Washington; on *Callianassa gigas* Dana

According to Sars (1913, p. 145), *Hersiliodes thompsoni* Canu (1888) and *Hersiliodes puffini* (Thompson) of Canu (1888) are also species of *Hemicyclops*. Sars merely says that these "can with full certainty be adduced to Boeck's genus." *H. puffini* (Thompson) was characterized as follows (Thomp-

son, 1887): "mandibles divided at apex into long slender spinose teeth. . . . first 4 pairs of swimming feet 2-jointed. . . . fifth pair swimming feet composed of 1 joint only with one side serrated. . . . the other having 2 serrated leaf-like spines. . . . first joint (of abdomen) doubly toothed at posterior angles." Canu (1888, p. 424) identified a young individual "au deuxième stade-copépod pourvue de trois paires de pattes" as belonging to Thompson's species, but on the basis of the single immature individual removed it to the genus *Hersiliodes*. Obviously this can hardly be construed as being clearly a species of *Hemicyclops*. For the present, therefore, we are not considering *Hersiliodes puffini* (Thompson) of Canu (1888) as belonging in *Hemicyclops*. *Hersiliodes thompsoni* Canu (1888, p. 422) was based on two immature females found on the body of *Callianassa subterranea* Montagu. In characterizing the family HER-SILIIDAE Canu says (1888, p. 406), "mandibules dépourvues de palpe." The genus *Hemicyclops* is characterized by the presence of well-developed mandibular palpi. This character may have been overlooked by the author of the family HER-SILIIDAE. There is still the statement that in *H. thompsoni*, "le repli pleural céphalique porte une ligne de petites épines courtes et très espacées." Whether this is a juvenile character or one of sufficient importance to separate the species from *Hemicyclops purpureus* cannot be determined.

KEY TO SPECIES OF HEMICYCLOPS BOECK

1. Abdomen (excluding genital segment and caudal rami) clearly 4-segmented. 2
1. Abdomen 3-segmented 4
1. Abdomen 3-segmented; genital segment somewhat transversely grooved. 5
2. Third segment of second antenna produced laterally and distally (fig. 18); segments 3 and 4 not greatly shortened. *H. pugettensis*, p. 181
2. Third segment of second antenna not produced. 3
3. Segments 3 and 4 of second antenna together less than half as long as segment 2
H. aberdonensis
3. Segments 2 and 4 of second antenna about equally long. *H. adherens*
4. Furcal rami 4 times as long as wide. *H. callianassae*
4. Furcal rami less than twice as long as wide. *H. purpureus*
5. Segment 3 of second antenna greatly produced on one side so as to extend distally beyond segment 4. *H. thysanotus*
5. Segment 3 not so produced. *H. americanus*

DESCRIPTION OF NEW SPECIES

Hemicyclops pugettensis sp. nov.

(Figs. 12-20; pl. 12, figs. 5, 6)

Female—

(Mm.)

Length over all, including furcal rami.....	2.66
Length of furcal setae.....	1.28
Greatest width	1.02
Length of 4 abdominal segments.....	0.68
Length of cephalothorax in middorsal line.....	0.68
Length of second thoracic segment in middorsal line.....	0.22
Length of third thoracic segment in middorsal line.....	0.30
Length of fourth thoracic segment in middorsal line.....	0.14
Length of egg string.....	1.08
Width of egg string.....	0.32
<i>Male</i> (proportions similar, but smaller in size)—	
Length over all, including furcal rami.....	1.82
Greatest width	0.76

Female.—Cephalothorax wider than long (pl. 12, fig. 5); lateral expansions of thoracic segments considerably produced, each side projecting freely for almost one-fourth of its entire width. Posterior border of dorsal shield broadly emarginate, leaving part of segment 4 and all of segment 5 exposed.

Genital segments trapezoidal (fig. 12; pl. 12, fig. 5), broader than long, widest anteriorly. Spermatophores large, about two-thirds as long as the genital segment, asymmetrically reniform, attached dorsally and just posterior to that of the ovisacs. Ovisacs slender, long ellipsoid, more than three times as long as broad, attached by a short pedicle at sides of widened part of genital segment (pl. 12, fig. 5).

Abdomen 4-segmented, depressed cylindrical; tapering somewhat distally (fig. 12); first and second segments about equally long, third and fourth successively shorter, the fourth about half as long as third, and half as long as wide.

Caudal rami almost 5 times as long as wide (fig. 12); diverging distally, each ramus provided with 4 setae of unequal length and a small spine on inner ectal margin (fig. 13).

First antenna 7-segmented, with numerous long, slender setae; fourth segment the longest, second the next longest, other segments equal or subequal in length (fig. 15).

Second antenna 4-segmented (fig. 18); sharply bent at an angle between segments 1 and 2. Segment 1 the longest, about three times as long as wide, with a long plumose seta at its distal ectal margin and with a fine seta and numerous hairs on its inner margin (fig. 18). Segment 2 longer than broad, widest distally, with numerous long hairs on its proximal half and a seta on its distal half (fig. 18). Segment 3 shorter than segment 2, produced along its outer margin so as to lie at side of segment 4, but not extending so far distally as 4; spinous on its outer margin; its distally produced portion having a heavy spine and a setose spine, and subdistally a long, coarse seta and a finer, shorter one (fig. 18). Segment 4 squarish, having distally 7 long, gently curved setae.

Upper lip a large flap between bases of second antennae and overhanging mandibles. Mandibles as in figure 17; mandibular palp exceeding mandible in size, as long as wide, having along distal border 8 spinelike setae. Lower lip an inflated circular area between bases of maxillipeds, partly filling a broad area between maxillipeds and first thoracic legs.

First four thoracic legs similar to one another. Setal formulae as follows (sp = spine; se = seta): Leg 1, exopodite: sp-0; sp-se; 2sp-6se; endopodite: se-0; se-0; 5se-1sp. Leg 2, exopodite: sp-0; sp-se; 2sp-7se; endopodite: se-0; 2se-0; 3se-3sp. Leg 3, exopodite: sp-0; sp-se; 2sp-7se; endopodite: se-0; 2se-0; 3se-3sp (including a long distal seta). Leg 4 (fig. 20), exopodite: sp-0; sp-se; 2sp-7se; endopodite: se-0; 2se-0; 2se-3sp. Leg 5 two-

segmented; basal segment reduced, with 1 seta; distal segment broad, with 4 setae and numerous fine hairs along outer margins (fig. 12).

Male.—Proportions of metasome similar to those of female (pl. 12, figs. 5, 6) but smaller (cf. measurements given above). Genital segment (fig. 14) broader than long, laterally inflated, sharply set off from abdomen, provided with foot rudiments on ventroectal border near posterior end of segment. Maxilliped as in figure 17; other appendages similar to those of female.

Holotype.—U.S.N.M. no. 71678; paratypes in the University of California.

Locality.—False Narrows, Puget Sound, Washington, on surface of *Callianassa gigas* Dana.

Systematic discussion.—*Hemicyclops pugettensis* is readily identified by (a) its greatly elongated urosomal segments (fig. 12) and (b) the shape and proportions of the penultimate segment of its second antenna (fig. 18). It differs from *H. callianassae* Wilson and *H. thysanotus* Wilson (pl. 12, figs. 3, 4) in having 4 abdominal segments instead of 3, and in the proportions of the segments of the second antenna.

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EXPLANATION OF PLATE

EXPLANATION OF PLATE

PLATE 12

(Photographs by Mr. John E. Gullberg, all $\times 21.5$)

Figs. 1, 2. *Clausidium vancouverense*, female with attached male

Fig. 1. In dorsal view.

Fig. 2. In ventral view.

Figs. 3, 4. *Hemicyclops thysanotus*

Fig. 3. Female, in dorsal view.

Fig. 4. Male, in dorsal view.

Figs. 5, 6. *Hemicyclops pugettensis*

Fig 5. Female, in dorsal view. Caudal setae omitted

Fig 6. Male, in dorsal view.



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**HOST-PARASITE RELATIONS
IN THE DISTRIBUTION OF
PROTOZOA IN TERMITES**

BY

HAROLD KIRBY, JR.

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HOST-PARASITE RELATIONS IN THE DISTRIBUTION OF PROTOZOA IN TERMITES

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HAROLD KIRBY, JR.

WENRICH (1935) has recently reviewed the problem of host distribution of entozoic Protozoa in general, discussing its bearing on evolution and genetic relationships. His long consideration of the question led him to support the view that a rigid limitation of parasites to particular species or related species is not the rule, so that generally there is no justification for seeking parallels in the evolutionary history of host and parasite. He finds no support for the view that the evolution of parasites has been influenced in a directive manner by the hosts. He remarks, however, that in the ophryoscolecoid ciliates there has been a striking degree of evolution within the group of ruminant hosts; but there seems to be no rigid host-parasite specificity, not even according to degree of host relationship, so that phylogenetic parallels cannot be drawn. He also directs attention to the marked degree of evolution of polymastigote and hypermastigote flagellates in termites and in the wood-eating roach *Cryptocercus punctulatus*, but he does not discuss this at any length. Undoubtedly, it provides a very significant instance of the evolutionary development of a group of entozoic Protozoa and at the same time is promising material for concomitant studies of the taxonomic differentiation of protozoan parasites and their hosts.

Metcalf (1929) states that the concomitant study of parasites and their hosts has attracted attention independently from at least six investigators during the past forty years: von Ihering (1891, 1902), studying parasitic worms, mainly of vertebrate hosts; Kellogg (1913, 1914), working with ectoparasitic Mallophaga and Anopleura of birds and mammals; Harrison (1911-1928), also discussing Mallophaga of birds and mammals, as well as parasitic worms in freshwater crayfish, and other parasites; Metcalf (1920-1928), concerned with opalinid ciliate Protozoa of frogs and toads; Darling (1925), believing that he could derive some evidence for the origin and distribution of races of man from the hookworms; and Ewing (1926), finding genetic significance in the pediculids of monkeys and man. Johnston (1913-1916) discussed trematodes and cestodes as indicative of genetic relationships between their Australian hosts and those in other regions. Baylis (1924) considered the problem of specificity in host distribution of nematodes; and Sandground (1929) investigated the bearing of the phenomena of age resistance and acquired immunity on the same question. Hegner (1928) recognized the importance of host-parasite data derived from study of Protozoa in their bearing on the evolutionary relationship of monkeys and man. In a somewhat different, but related, problem Emerson (1935*b*) found that the distribution of termitophiles in the nests of certain species of *Nasutitermes* in British Guiana served as an indicator of speciation.

Such studies as these often contribute toward the solution of problems of taxonomy and genetic relationship, and sometimes at least the data may be used to aid in forming judgment on contemporary and historical aspects of geographical distribution. In the latter manner of using the data, Metcalf has been particularly prominent; the student interested in the general problem should refer to his 1929 review.

Although he himself had carried on no investigations of the Protozoa of termites, Metcalf recognized their value for such studies, stating (1929, pp. 20-21) that "probably the finest groups for host-parasite studies are the termites and the flagellates living in their intestines." Other groups of Protozoa, as well as other entozoic animals, besides those that have already been considered from this standpoint, offer opportunities for such studies. The flagellates in termites, however, have certain unique advantages. The writer has studied the Protozoa of termites for the past ten years, and has had in mind this significance of the group, but no more than incidental statements bearing upon it have been made. A large amount of descriptive work is necessary before general conclusions will be thoroughly justified and firmly supported. Although this has by no means been completed, it seems desirable to make a preliminary presentation of the problem at this time.

Baylis (1924) quotes Caullery's statement that "there can be no possibility of regarding parasites as forms specially devised by Providence as complementary to the life of particular hosts." One of the closest approaches to that possibility, however, is made by the flagellates of termites. Every species of four of the five families of Isoptera—*Mastotermitidae*, *Hodotermitidae*, *Kalotermitidae*, and *Rhinotermitidae*—contains flagellates in the hind gut, and these are present at all stages of the life history except the eggs, the youngest instars, the specialized reproductives of certain species, and in each member of the colony preceding or immediately following a molt. The diversity of these flagellates is remarkable, and most of them, constituting by far the most highly developed *Mastigophora*, belong to groups represented nowhere else except in certain roaches. A few of the flagellates resemble certain of those that occur in animals other than termites and these roaches, but the great majority constitute a unique association.

The amount of published data on the Protozoa of termites is very limited, when compared with what there is to be done. The writer, however, has a large amount of still unpublished information on which to base the conclusions stated in this paper. This has been derived from the study of material sent by correspondents; that collected in Central America in 1925; and that recently prepared in Egypt, British East Africa, South Africa, Madagascar, Reunion, Mauritius, and Java during tenure of a Guggenheim fellowship. Smears showing the faunas of about a third of the approximately 430 known termites of the four lower families, all of which doubtless contain flagellates in abundance, are in this collection. Besides those termites whose entire faunas are therein represented, a little is known of the flagellates in about forty others. This knowledge has been derived from incomplete published descriptions and from specimens preserved in alcohol. The members of the

TABLE 1

CLASSIFICATION OF TERMITES

(Giving the approximate number of species known and the number examined for Protozoa. The figures in the second column give the number in which the fauna is quite well known, except those in parentheses, which give the additional number of termites in which a little is known of it.)

Classification	Number of species	Number examined
Four lower families	427	141 (36)
Family I. Mastotermitidae	1	1
Genus Mastotermes	1	1
Family II. Hodotermitidae	21	4 (2)
Genus Hodotermes	15 (?)	2 (2)
Genus Anacanthotermes	6	2
Family III. Kalotermitidae	264	107 (27)
Lower genera	17	10 (1)
Genus Archotermopsis	1	1
Genus Zoötermopsis	3	3
Genus Hodotermopsis	2	(1)
Genus Stolotermes	6 (?)	3
Genus Porotermes	5	3
Kalotermes group		
Genus Kalotermes	247	97 (26)
Subg. Kalotermes s. str.	49	20 (5)
Subg. Neotermes	75	12 (11)
Subg. Paraneotermes	1	1
Subg. Rugitermes	10	2
Subg. Cryptotermes	34	14 (2)
Subg. Planocryptotermes	1	1
Subg. Procryptotermes	6	3
Subg. Eucryptotermes	1	0
Subg. Calcaritermes	8	3
Subg. Glyptotermes	49	13 (8)
Unassigned to subgenus	28 (13 new?)	28
Family IV. Rhinotermitidae	141	29 (7)
Genus Psammotermes	6	3
Genus Leucotermes	26	4 (1)
Genus Reticulitermes	14	6
Genus Stylotermes	1	0
Genus Coptotermes	46	11 (2)
Genus Prorhinotermes	13	3
Genus Termitogeton	2	(1)
Genus Parrhinotermes	5	(2)
Genus Rhinotermes	27	2 (1)
Subg. Rhinotermes s. str.	9	(1)
Subg. Schedorhinotermes	18	2
Genus Serritermes	1	0
Family V. Termitidae	about 1200	about 40 (51)*

* The latter figure is the number examined by others, including Cleveland, as reported by him (1923), less those also examined by the writer.

fifth family, the Termitidae, which includes nearly twelve hundred species, in general have few or no Protozoa; but significant information has been obtained from some of the species investigated.

Apart from the genus *Retortamonas* (= *Embadomonas*), one species of which was recorded by the writer from one of the Termitidae, *Amitermes beaumonti*, all the flagellates known in termites belong in the two highest orders, Polymastigida and Hypermastigida. The writer now recognizes in termites 30 genera comprising 133 species of the former order, and 18 genera with 63 species of the latter—totaling 48 genera and 196 species. Actually, the number of species in the material in his collection will greatly exceed that figure, and doubtless a number of new genera will be added. All but the genus *Chilomastix*, besides *Retortamonas*, are represented in the four lower families; only *Trichomonas* and *Monocercomonas* (= *Eutrichomastix*) are known to occur in both groups.

Individual faunas of flagellates in the more primitive termites comprise from two to as many as ten species, but the extremes in number are unusual. Sometimes there are three species of a genus in one host, more frequently there are two, but usually there is only one. More remarkable than any one known fauna of flagellates in termites is that in *Cryptocercus punctulatus* reported by Cleveland (1934). In that wood-feeding, wingless roach there are nine genera of hypermastigotes, with twenty species, and three genera of polymastigotes, with five species, as well as a gregarine and a coccidian. In *Trichonympha*, the only hypermastigote genus also found in termites, there are seven species; in another genus, *Barbulanympha*, there are four. The discovery of this fauna is of great significance in connection with studies of the phylogeny of the flagellates of termites.

Some questions that suggest themselves in connection with the concomitant study of these flagellates and their hosts are: (1) What is the degree of host-parasite specificity in present distribution? (2) Are the faunas of all individual termites of a species identical? (3) In what measure can the flagellate faunas be of assistance in distinguishing species? (4) Are the faunas of more primitive termites of more primitive character? (5) In what measure has evolutionary development of the flagellates taken place in termites? (6) To what degree do the flagellate faunas serve as indicators of the relationship of the hosts? It is not yet possible to give a final answer to all these questions, especially the last two, but the paths to be followed in further exploration have at least been clearly blazed.

1. As in other Protozoa and in parasitic nematodes, the degree of host-parasite specificity varies in different genera and species. Many species are known from one host only, but as more flagellate faunas of termites become known, the tendency probably will be relatively to reduce rather than to increase this number. It is clear that occurrence of a flagellate in a different termite cannot in itself be used as evidence for specific differentiation of the protozoan; the most flagrant violation of this principle is Zelif's (1930) treatment of the genus *Oxymonas*. Certain species are found in many hosts. Fifteen species of *Foaina* have been distinguished by the writer on the basis of

morphological characteristics, and thirty-three termites harbor one or two, one three, and one four of these. Eight species of the flagellate were found in one host each, three in two, but at the other extreme one species occurs in fifteen from various parts of the world. Four of thirteen species of *Devescovina* were each recorded from one host only, whereas one, *D. lemniscata*, was found in seven, from the widely separated regions of Central America, Australia, Fanning Island, and India. *Trichonympha agilis* probably occurs in all species of *Reticulitermes*, but has not been found outside of that genus. The small *Tricercomitus* which probably occurs in most, if not all, species of *Kalotermes* seems to be one species, *T. divergens*, so far as can be learned from its minute anatomy.

To the writer it seems improbable that cross infection among species of termites has been an important factor in determining the present distribution of flagellates, at least below the Termitidae. Furthermore, the unique characteristics of almost all the flagellates, which have no close relatives in animals other than the group under consideration, indicate that the faunas of more primitive termites do not in any large measure include recent acquisitions from other groups of arthropod or other hosts. The question is fundamental to correlative phylogenetic studies, and more experimental work as well as more study of the faunas is necessary before one would be justified in excluding the possibility.

The habits of termites and the life histories of the flagellates are such that one host species is unlikely to acquire an infection from another. The social isolation of the communities of more primitive termites is conspicuous as in few other groups of free-living animals, even when, as is sometimes true, two or three species occur in one log. The transmission of intestinal Protozoa from one host to another is usually accomplished by cysts, and where cysts do not occur, direct contact or ready means of dispersal are necessary. It is doubtful, however, if cysts or even resistant stages are formed in most of the flagellates in termites. More investigation of that possibility is needed, and the subject cannot be fully discussed here. It is important to distinguish between true cysts and the rounded-up forms, sometimes surrounded by an abnormal, clear, uneven peripheral layer, which are occasionally encountered on slides and are caused by technical treatment or a poor condition of the flagellates before fixation. Trager (1934) found that rounded-up, resistant forms of *Tricercomitus termopsidis*, which were able to resist drying for one or two days, were formed in cultures on solid media. Duboscq and Grassé (1934b) have described cysts of *Tricercomitus* and *Hexamastix* in *Kalotermes flavicollis*, and further study of the situation in these small flagellates is desirable. Duboscq and Grassé believe that *Mesojoenia* in *K. flavicollis* is a postdivisional or precystic stage of *Joenia annectens*, and that the occasional intracytoplasmic position of the bundle of flagella in *Joenia* is an accompanying phenomenon of encystation. This introversion of flagellar bundles has been observed by Grassi (1917) and the writer (1926) in *Staurojoeninina*, and by Cleveland (1934) in several of the roach hypermastigotes. In *Staurojoeninina* there is no evidence that this condition is associated with encystation, though in *Barbula-*

nympha it occurs during the development of resistant stages. As yet there is no conclusive evidence for the true encystation of any of the flagellates in termites, and it seems unlikely that any will be found except perhaps in some small polymastigotes.

Except for those found in *Lophomonas*, the only occurrences of true cysts in hypermastigotes were reported by Cleveland in *Trichonympha* and *Macrospironympha* in *Cryptocercus*. There is no doubt about these. Of the other hypermastigotes in the roach, *Barbulanympha* and *Rhynchonympha* are said to form resistant stages, with rounding up and loss of extranuclear organelles, but there is no cyst wall. *Leptospironympha*, *Eucomonympha*, and *Prolophomonas* were not reported to undergo similar changes. One of the three species of the polymastigote *Saccinobaculus* is said to form cysts, but as figured they suggest the rounded forms of some polymastigotes of termites. It is probable that the habit has been lost in most of the flagellates of termites because the faunal relationships among members of termite colonies, which differ from those in *Cryptocercus* (Cleveland, 1934, pp. 208–209), have made such a stage unnecessary. There is facile transmission of active trophozoites from termite to termite, probably by proctodaeal feeding, though the exact manner could not be determined by Andrew (1930). The young termites of a new colony are infected in the same manner by the parents, which usually, at least (Emerson, 1935*b*, p. 392), were members of the same colony. Thus, there is the closest possible continuity in the flagellate faunas of termites, and infection of one species from another could occur only as a result of an exceptional circumstance. The chances that such an exceptional event would lead to the establishment of a new infection of the species, or even of a new member of the fauna, are small. Light and Sanford (1927) found that no establishment of unaccustomed Protozoa took place even when defaunated nymphs of *Porotermes* were kept in close association with *Zoötermopsis angusticollis*, *Kalotermes* (*Neotermes*) *malatensis*, and *Reticulitermes hesperus*, and even feeding the intestinal contents was unsuccessful. But Cleveland (1934) did accomplish transfaunation by feeding the Protozoa of *Zoötermopsis* to *Cryptocercus*, and the Protozoa of the latter species to the former.

There seems to be no resistance to cross infection. The internal environment of the intestine of one wood-feeding termite, with the possible exception of those that feed on particular types of wood, probably does not differ essentially from another, at least not in a measure sufficient to determine the survival of Protozoa of particular species. The experiments of Light and Sanford (1927, 1928), in which Protozoa of one species were injected through the anal aperture into defaunated nymphs of another species, showed that survival of *Zoötermopsis* flagellates is possible in *Porotermes* for at least twelve days, and in *Kalotermes hubbardi* for at least one hundred days; that is, as long as the termites used in the experiments survived. Furthermore, Cleveland (1934) found that transfer of the fauna of *Cryptocercus* to *Zoötermopsis*, or that of *Zoötermopsis* to *Cryptocercus*, is easily accomplished, and multiplication takes place.

Certain groups of flagellates occur only in certain groups of termites,

although others are widespread. The faunas of the different species of termites in a region may be sharply distinguished from one another; or, if there are certain species in common, the explanation can be sought in host relationships and is in agreement with the distribution of those flagellates in related but geographically separated species of termites.

2. It was thought at one time that the faunas of all termites of a species are identical. Further study shows that this statement must be modified, although it is true in general. Sometimes certain flagellates recorded from a species are absent not only from individual termites but even from all members of a colony. Cleveland (1934) found this to be true in his study of the faunas of *Cryptocercus punctulatus* from the Appalachian region and the Pacific states. Colonies of *Kaloterme immigrans* have been found which lack *Trichonympha*. Oxymonads were at least so scarce in certain colonies of Kalo-termitinae collected in Africa and Madagascar that they were not noted at all, whereas in other colonies of the same species they were abundant. *Trichonympha campanula* was found in all three species of *Zoötermopsis*, but *T. collaris* and *T. sphaerica*, present in *Zoötermopsis angusticollis* and *Z. nevadensis*, were absent from the one colony of *Z. laticeps* examined. It is necessary to examine at least several colonies, preferably from different regions, before it can be concluded safely that a flagellate is absent from the species.

The explanation of this irregularity in distribution doubtless is to be found in the occasional absence of certain flagellates from the pair which start a new colony, and it is not difficult to surmise how such an event might be brought about. Nevertheless, uniformity in the composition of the faunas is the rule, a testament to the effectiveness of the methods of transmission among the members of a colony.

3. Among the more primitive termites, particularly, it is usual to find differences between the intestinal faunas of different species, but identical faunas occur in different termite species of certain groups. The fact, therefore, that the flagellate faunas are identical does not necessarily indicate that the hosts belong to the same species. At most, such identity offers a suggestion to aid a decision reached by study of the taxonomic characteristics of the termites. It may have a bearing on the evaluation of supposed specific distinction based on such uncertain characteristics as size.

When the faunas are markedly different, however, in greater degree than in the absence in one fauna of one or two flagellates, it is a strong indication for specific differentiation of the hosts. In some termites the first clue to specific distinction has been a difference observed in the flagellate faunas. The flagellates usually provide a ready means of distinguishing nymphs in a region where both the termites and their faunas are known, although certain species of termites, as *Zoötermopsis angusticollis* and *Z. nevadensis*, *Reticulitermes hesperus* and *R. tibialis*, and probably various species of *Coptotermes* and *Leucotermes*, cannot be distinguished in this way.

4. There is no correlation between primitiveness of the flagellate fauna and primitiveness of the hosts. Some of the most advanced hypermastigotes occur in *Cryptocercus*, *Mastotermes*, and in the more primitive Kalotermitidae.

Flagellates which may be considered as primitive, as *Tricercomitus*, *Tritrichomonas*, *Trichomonas*, *Hexamastix*, and *Monocercomonoides*, are widely distributed. Hypermastigotes predominate in the faunas of *Cryptocercus*, termites more primitive than *Kaloterme*s, and in the Rhinotermitidae. In *Kaloterme*s, with its many subgenera, comprising more than half the termites of the four lower families, polymastigotes predominate, often composing the entire fauna.

5. A comparative study of the protozoan faunas of termites and roaches supports the hypothesis, suggested by Cleveland (1934), that evolutionary development of certain groups of the flagellates took place in the ancestral insects from which termites and roaches developed, so that they passed into representatives of both groups. Few roaches have habits and protozoan faunas comparable to those of the more primitive termites. Apparently the only example among those now well known is *Cryptocercus punctulatus*. The hypermastigote *Lophomonas*, however, which is found in cockroaches, seems to be a relic of this fauna. This hypothesis is, of course, highly speculative, but it is better supported by the evidence from present distribution of the flagellates than is any other suggestion concerning their origin.

Cleveland (1934) believes that if the Protozoa of *Cryptocercus* did not come from some extinct blattoid ancestor, they may have lived in the soil. It seems to the writer that this possible origin can justly be excluded from consideration. Apart from the extreme improbability that any similar forms existed in the soil, it is unlikely that they would have passed into both termites and roaches, alone among animals, in such a manner as to be distributed as they now are. That the ancestral origin was from free-living flagellates, though probably by way of earlier entozoic habitats, cannot be disputed, but that development has since taken place in an entozoic environment and that that environment has been the intestine of primitive termites, primitive roaches, or their ancestral arthropods, is suggested by the facts.

Emerson (1935*b*), in a review of Cleveland's monograph, states that he recognizes a possibility that *Cryptocercus* picked up its Protozoa from termites in later stages of its evolution. This is no more probable than that termites have picked up flagellates from one another, the reasons for doubting which, as a general principle, the writer has already given. The possibility cannot be excluded; though it seems to the writer that the evidence is against it.

The genus *Trichonympha*, which is present in *Cryptocercus* and is widely distributed in termites of the families Hodotermitidae, Kalotermitidae, and Rhinotermitidae, is particularly interesting with respect to its probable antiquity. Among termites, the genus has been found in forty-five hosts of ten genera or subgenera, and among those that have been studied for detailed taxonomic characters, fourteen species have been distinguished. As mentioned above, *Cryptocercus punctulatus* contains seven species. *Trichonympha* is not closely related to other genera of hypermastigotes; there is a gap between it and existing genera, even the somewhat similar *Pseudotriconympha*, which has a restricted distribution. Its wide distribution in termites suggests its antiquity and relative constancy in characteristics, apart from speciation.

The genus *Trichonympha* probably existed in late Paleozoic or early Mesozoic times, when roaches and termites are believed to have diverged from the ancestral blattoid stem. Outside of termites, a group of species has persisted in *Cryptocercus punctulatus*. These species all possess a nuclear sleeve, which is not present in the species of *Trichonympha* in termites. They also differ in having retained the capacity for encystation. The possibility of recent origin of roach trichonymphs from termites is unlikely because of these and other distinctions, as well as the factors of distribution of the faunas, as is discussed above. Certainly the differences in morphology eliminate the possibility that existing *Reticulitermes* or *Termopsis*, which sometimes occur in the same logs with *Cryptocercus*, were the source.

Staurojoenina, which has a more restricted distribution in termites of the *Kaloterme*s group only, is somewhat related to the genus *Idionympha* in the roach. (It is noteworthy that *Trichonympha* and *Staurojoenina* have never been found in the same species of termite.) Various Holomastigotidae in termites are related to genera in *Cryptocercus*. Among the Holomastigotidae in termites, *Spironympha*, *Spirotrichonympha*, *Holomastigotes*, and *Holomastigotoides* are represented in widely separated groups, so that the situation is comparable to that in *Trichonympha*.

The evidence, then, derived from the relationship of the flagellates of *Cryptocercus* to those of termites, and faunal distribution of the flagellates in termites, points to the conclusion that most hypermastigotes, at least, underwent much of their remarkable evolution before termites and roaches diverged from the insects ancestral to the two groups. Possibly among these ancestors were wood-boring insects with habits resembling those of *Cryptocercus*.

But the situation in many of the polymastigotes is different. Except for *Hexamita* and *Monocercomonoides*, which are found in various animals, the only polymastigote of *Cryptocercus punctulatus* is *Saccinobaculus*. As Cleveland (1934) has shown, this is related to the pyrsonymphid and oxymonad flagellates of termites, so much that the three groups may all be included in the Pyrsonymphidae. There is no *Saccinobaculus* in termites, and it has certain more primitive features than either of the groups in termites.

These two groups, the Oxymonadinae and Pyrsonymphinae, have a restricted distribution in termites. The Oxymonadinae, which have developed to a high degree the rostellum as an organ of attachment, occur in many termites, but have been found only in the genus *Kaloterme*s, where the subfamily is represented in most species. By far the most frequent is the uninucleate genus *Oxymonas*, but a multinucleate condition has developed in the genera *Proboscidiella*, *Microrhopalodina* and *Kirbyella*. One of the more advanced forms, a species of *Proboscidiella*, occurs in *Kaloterme*s *occidentis*, which is considered to be a very primitive species of the genus; whereas another species of *Proboscidiella* occurs in the more advanced subgenus *Planocryptotermes*. Therefore it is probable that oxymonads have existed in more primitive genera, although they have not been found in any of those now existing that have been examined.

The Pyrsonymphinae, in which the four flagella have all become adherent

to the body, and in many instances have doubled the number, as a precocious predivisional development, to eight, have been found only in the genus *Reticulitermes*, where there are several species in each of the termite species so far examined. The restriction of these unique flagellates to those termites only, coupled with their frequency and wide distribution in them, suggests their evolution, probably from more primitive relatives of the oxymonads, in the termites directly ancestral to *Reticulitermes*.

Trichomonas and related genera are widespread in termites, being not infrequent in Termitidae as well as lower families, but in the Kalotermitidae certain advanced types have evolved, as the large *Trichomonas termopsidis* (from the four-flagellate type), *Pentatrichomonoides* (from the five-flagellate type), and *Pseudotrypanosoma* (from the three-flagellate type).

There are two groups, the Devescovininae and Calonymphidae, that are of exceptional interest in this connection. For studies of the evolutionary development of certain series these two groups, with the Pyrsonymphidae, are the most significant, for in them there is a graded series of forms that exist at present, whereas in hypermastigotes many intergradations are unknown and their discovery is not very likely.

The Devescovininae exist mainly in the *Kalotermes* group of termites. Outside of *Kalotermes*, devescovinids have been found only in *Mastotermes*, which has a species of *Metadevescovina*; *Hodotermes*, which has *Gigantomonas*; and *Anacanthotermes*, in which there are two species, one each of *Devescovina* and *Pseudodevescovina*. Of the ninety-seven termites of the *Kalotermes* group that have been examined thoroughly, only five or six species of the subgenus *Kalotermes* s. str., *K. immigrans*, *K. lighti*, *K. platycephalus*, *K. emersoni*, and *K. pacificus*, as well as *K. tabogae*, if that is separated from the last species, are known to lack at least one representative of the subfamily of flagellates. The occurrence of two or three species in a host is frequent; a number have four, and one at least has five. The diversity of species and genera seems to indicate a large amount of more recent evolutionary change than that of the Trichonymphidae and certain other hypermastigotes.

The origin of the Devescovininae seems to have been from the *Monocercomonas* (= *Eutrichomastix*) type of flagellate, which occurs in various animals and has been found in certain termites, though it is by no means so widespread in them as is *Monocercomonoides*.

The multinucleate Calonymphidae, with unit mastigonts ranging in number from eight to more than four hundred, and in *Snyderella* to several thousand, are also limited mainly to the *Kalotermes* group of termites. The only exception to this, among those now known, is the form named *Stephanonympha dogieli* by Bernstein (1928), in *Anacanthotermes murgabicus*. The status of that organism is uncertain, and will remain so unless further study is made of the flagellate itself. It does not belong to the genus *Stephanonympha*, but apparently is a calonymphid. The Calonymphidae are not so widespread as the Devescovininae, having been found in only about two-fifths of the termites of this group, and they are less highly diversified in species and genera. *Calonympha* and *Stephanonympha* are the common genera. *Snyderella* has been

found in one host species only, *Coronympha* in five, and *Metacoronympha* in four. The last two calonymphids have certain more primitive characteristics than the others, the mastigont having all the structures of a devescovinid flagellate, suggesting an origin from that type. So far, these two genera are known only in certain American species of the subgenus *Kaloterme*s s. str., the same species which are the only ones of the *Kaloterme*s group so far critically examined from which devescovinids seem to be absent. Nothing approaching the amount of polymerization in the Calonymphidae is known in any other flagellates, though a limited amount of development in that direction has also occurred in the Hexamitidae, Oxymonadinae, and *Polykrikos*. It is noteworthy that, except for *Polykrikos* and the doubling of nuclei and mastigonts in the Hexamitidae, this type of development has apparently taken place only in the *Kaloterme*s group of termites, apart from one possible, but uncertain, occurrence of it in *Anacanthoterme*s.

6. Finally we come to the question of the degree to which the flagellate faunas serve as indicators of the relationship of the hosts. In certain groups, it would generally be possible to assign the termite correctly from a knowledge of its flagellate fauna alone. To mention the most obvious examples of applying this principle, almost all unknown members of the Kalotermitinae could be placed in that subfamily; one of the Hodotermitidae could be recognized as belonging to that family; all members of the genus *Reticuliterme*s could be correctly assigned, and it could be stated whether or not a termite belongs in the Rhinotermitidae other than *Reticuliterme*s. The flagellates of these termites bear out the relationship indicated by their classification in the Isoptera.

The faunas may also indicate that a supposed relationship is incorrect. Banks and Snyder (1920) placed *Leucoterme*s in the subfamily Termitinae, that is, among the present Termitidae and apart from the subfamily Rhinotermitinae, which contained the other present Rhinotermitidae. A consideration of the Protozoa alone, however, would have suggested that it belonged in the Rhinotermitinae. According to Sjöstedt (1925), the genus *Leucoterme*s, for which he gives *L. tenuis* as the type, contains as one species *Leucoterme lucifugus*. Yet this termite, which other students place in *Reticuliterme*s, has a flagellate fauna differing profoundly from that of *L. tenuis*. All species of *Leucoterme*s have faunas related to those of *Coptoterme*s, *Rhinoterme*s, *Psammoterme*s, and all other Rhinotermitidae except *Reticuliterme*s, which is unique among termites in its flagellates.

The flagellate faunas support the generic separation of *Anacanthoterme*s from *Hodoterme*s, as is done in Sjöstedt's (1925) monograph. The four or five species of *Hodoterme*s (both the subgenera, *Hodoterme*s s. str. and *Microhodoterme*s) that have been examined have faunas that are much alike, if not identical. Those in the two species of *Anacanthoterme*s examined, *A. murgabicus* from Turkestan and *A. ochraceus* from Egypt, are also alike. The two types of fauna, however, differ greatly from each other.

The many instances in which the flagellate faunas throw light on relationships cannot all be mentioned here. Final conclusions with respect to the

significance of much of the data await more detailed study both of the faunas and of the phylogeny and taxonomy of the Isoptera. This is particularly true of the Kalotermitinae, where somewhat more than half the known termites of the lower families have been grouped in the genus *Kalotermes*, under ten subgenera. As noted above, there are certain groups of flagellates, the Devescovichinae, Calonymphidae, and Oxymonadinae, which are mainly restricted to *Kalotermes*. They are distributed throughout the group, but certain of them are characteristic of certain subgenera. These flagellates may be of value in contributing to final conclusions about ultimate designation of genera in this group of termites. *Trichonympha*, which is so widespread in termites, is of little value for such purposes; but the other hypermastigotes, of which *Staurojoenina* is the one most frequently found, seem also to be significant. Hypermastigotes other than *Trichonympha* and *Staurojoenina* occur in only 4 species of *Kalotermes* out of the 105 investigated. When, as in the species *Kalotermes* (*Paraneotermes*) *simplicicornis*, there are such unique forms as *Kofoidia* and *Hoplonympha*, a marked separation of that termite from others with which it has been associated taxonomically is suggested.

The Oxymonadinae are distributed throughout the large genus *Kalotermes*, and have no genetic significance within the genus. The more advanced types, *Proboscidiella* and *Kirbyella*, occur in such markedly separated termites that they cannot be used for the purpose. In the Calonymphidae and Devescovichinae there are certain suggestive relationships, but a greater range of detailed study is necessary before any conclusions can be drawn. Certain advanced types of Devescovichinae are particularly characteristic of certain subgenera; for example, *Macrotrichomonas* in *Glyptotermes* and *Calcaritermes*; but the same species occurs in *Kalotermes contracticornis* of Costa Rica and *K. (Glyptotermes) parvulus* of the Gold Coast, as well as certain Java and Ceylon *Glyptotermes*, and one species occurs in *K. (Paraneotermes) simplicicornis*. Advanced devescovichids of the genera *Caduceia*, *Pseudodevescovina*, and an undescribed monotypic genus have been found only in species of *Neotermes*. The *Coronympha* type of calonymphid has been found only in certain species of the subgenus *Kalotermes* s. str.

In the Rhinotermitidae the presence of *Pseudotrichonympha*, not found in other termites, in all genera and species except *Reticulitermes*, suggests a genetic relationship among them, and, as stated before, a unique position for *Reticulitermes*. Usually *Pseudotrichonympha* is accompanied by *Holomastigotoides*, which elsewhere among termites occurs only in Hodotermitidae, and *Spirotrichonympha*, which has species in Hodotermitidae, certain Kalo-termitidae, and *Reticulitermes* as well.

The protozoan infections of the Termitidae are comparable to those more usual in insects, in that there are few species and the number of individuals is relatively small. *Trichomonas* is the most frequently encountered flagellate, and is widely distributed, but it does not develop the large size or relative complexity of certain Trichomonadinae in lower termites. *Nyctotherus* has been found in certain Termitidae, as well as in *Mastotermes*, *Hodotermes*, and a species of *Neotermes*. Of greatest interest are the amoebae in certain groups.

Small amoebae occur in most species of *Amitermes*, and both small and large amoebae have been found in termites of the *Mirotermes-Cubitermes* group. These termites are related in their position in classification and in their habits. Amoebae have been found in no termites of the lower families except *Hodotermes* in Africa.

Most of these amoebae have not yet been studied in detail. Those in one Panamanian and five Californian species of *Amitermes*, and in two species of *Mirotermes*, were described by the writer (1927, 1932b). A number of species of *Amitermes* were examined in East and South Africa, and it was most interesting to find that they consistently contained the same type of protozoan fauna, composed apparently of the same species as those found in the Californian termites. *Trichomonas* was practically always present, small amoebae usually, and in many species of *Amitermes* was found *Nyctotherus* of the species *silvestrianus*, which has not been noted elsewhere than in termites of that genus.

In termites of the *Mirotermes-Cubitermes* group, amoebae of size comparable to those of the Panamanian *Mirotermes* were usually found, together with a small *Trichomonas*, so consistently that it seems possible that most if not all termites of those groups will be found to contain them. In the species from Panama there were marked differences between the faunas of the two hosts, *M. panamaensis* containing two species of amoebae, and *M. hispaniolae* lacking these but containing three others. In the Panamanian species the amoebae were of value in confirming the systematic distinction of the termites, and it is possible that among the numerous African species the amoebae will prove to be of equal value. Large amoebae were also found in *Capritermes capricornis* of Madagascar.

In many other African Termitidae examined, including species of *Nasutitermes*, *Microcerotermes*, and various fungus growers, no amoebae were found. In some there were a few small flagellates, and occasionally *Nyctotherus* or a gregarine. Most of the African species of *Nasutitermes* examined were devoid of Protozoa, as were several in Panama, the exceptions containing a few minute flagellates. Cleveland (1923), however, reports having found Protozoa in a species of *Nasutitermes* from the Belgian Congo. Two workers of *Nasutitermes* (*Angularitermes*) *nasutissimus* collected by Emerson in Kartabo, British Guiana, preserved in alcohol, and sent to the writer for examination, were found to contain oöcysts of *Eimeria*.

A complete and accurate account of all the Protozoa of termites and wood-eating roaches of the world would be very valuable material for consideration by the student of the facts of evolution. For this reason, as well as for its cytological value, taxonomic and morphological work on the group makes a contribution to biology greater than the mere addition of new species to protozoölogical literature. Unfortunately, however, much of the literature is inaccurate and consequently misleading, so that reëxamination of the organisms is necessary.

The bearing of the flagellate faunas upon the taxonomy and genetic relationships of termites, which is discussed in this paper, is not the problem of

greatest significance, though the principle involved is important to biology. In the association between termites and their flagellates it is possible to work out many features of development in a group of organisms that have lived for a great period of time in an environment that probably is of as great uniformity as exists anywhere in nature, where they are subjected to a minimum of environmental stimulus. For the requirements of their existence the simpler structures function as well as the more complex, and various characteristics seem to have developed independently of competitive factors. Apparently no form of selective adaptation, other than elimination of defects, is involved.

The probable inoperativeness of natural selection also is indicated by studies in other groups, as dinoflagellates, Tintinninoinea, Ophryoscolecidae, and Salpidae. Metcalf (1928) has discussed the situation in the Opalinidae, Ophryoscolecidae, and Salpidae, concluding that evolutionary change in them seems to be the result of internal factors without external control, an expression of the germ plasm through mutations in a seemingly orthogenetic manner. Wenrich's (1935) consideration of the situation in entozoic Protozoa in general led him to the belief that "new species are primarily of mutational origin, rather than the direct effect of the host in a Lamarckian manner." It is now generally agreed that new characteristics are the result of germinal mutations; the problem is to explain the occurrence of these. Kofoed (1930), in a thoughtful analysis, has considered the factors in the evolution of the Tintinninoinea. These pelagic ciliates occur for the most part in the sea, and have undergone evolution, manifested by the loricae, into 705 known species, placed by Kofoed and Campbell (1929) in 51 genera and 12 families. After excluding isolation as a factor, and pointing out the lack of differential survival value in the characters of the loricae which serve for distinction of species, he seeks a direct environmental agency, and suggests that this may be solar radiation.

Speculation concerning the reason why there has been such a remarkable amount of genetic change in the flagellates of termites and *Cryptocercus*, as compared with that in other flagellates, is tempting. The environment has been more sheltered and uniform than that of most habitats; the amount of evolutionary change has been much greater than in any closely related organisms. Could it be that the very absence of variable environmental influences has itself passively favored this behavior of the germ plasm?

NOTES ON TABLE 2

Several genera of incorrect or uncertain status have been omitted from this tabulation. The writer's studies of *Foaina gracilis* Janicki indicate that *Janickiella* Duboseq and Grassé and *Paradevescovina* Kirby are synonyms of *Foaina*. *Ditrichomonas* Cutler is a synonym of *Trichomonas* (Cleveland, 1934, p. 267). *Diplonympha*, described by Grassi (1917) as having two nuclei in a mastigont, actually has but one, like *Stephanonympha*, of which it is probably a synonym. (Material of this flagellate has been available for study through the kindness of Professor Silvestri). *Metastephanonympha*

seems to be a synonym of *Stephanonympha*. Mello and Brito (1929) distinguish it from that genus for no stated reason except the presence of two flagella in a mastigont instead of four, and there is much reason to doubt the correctness of their statement. Probably all Calonymphidae have four flagella in a mastigont. *Eulophomonas* is of doubtful status; Cleveland (1934, p. 289) could find no flagellate in *Kaloterms flavicollis* answering to Grassi's description. It is quite possible that *Torquenympha* Brown is a synonym of *Microjoenia*. *Mesojoenia* Grassi probably is a synonym of *Joenia*, but whether *M. decipiens* is a synonym of *J. annectens*, as Duboseq and Grassé (1933, p. 435) contend, remains to be decided. *Spirotrichonymphella* Grassi, although listed separately here, may be a synonym of *Holomastigotes*. *Pseudotrichonympha pristina* Cutler, from *Archotermopsis wroughtoni*, belongs in a different, probably a new, genus. This is obvious from the description (Kirby, 1932, p. 424), and it was separated from *Pseudotrichonympha*, but not named, by Cleveland (1934, p. 272) after study of specimens from the same host species. *Myxomonas* Dogiel probably is a synonym of *Gigantomonas*. Other omissions have been explained in previous publications.

Parajoenia belongs in the Devescovininae and not the Hypermastigida. Duboseq and Grassé (1933) place it where Janicki did, in the Joeniidae, and regard it as transitional between the devescovinid flagellates and *Joenia*. The writer (Kirby, 1937) has studied living material from *Kaloterms* (*Neoterms*) *connexus* of Hawaii, as well as stained material, and has determined that there are three long anterior flagella, as in other devescovinids. The numerous anterior flagella described by Janicki are adherent spirochaetes.

A flagellate similar to *Devescovina elongata* Bernstein is present in *Anacanthotermes ochraceus*, but it has a cresta, not noted by Bernstein, and one of the four flagella is a trailer, though no stouter than an anterior flagellum. *D. elongata*, therefore, is truly a species of *Devescovina*, contrary to the opinion of Duboseq and Grassé (1929) that it is a species of *Polymastix*.

Opisthomitus occurs in many species of *Kaloterms*, but only the one from which it has been reported is listed, the double plus indicating the many additional hosts.

Of the twenty-one species given in table 1 as the number of Hodotermitidae known, six or seven have been examined, but in table 2 have been recorded only the faunas of those four concerning which there is no question.

Some genera placed in synonymy previously by various writers are recognized in table 2 as distinct. *Caduceia* França and *Metadevescovina* Light (the latter doubtfully) are so treated by Duboseq and Grassé (1927), but the writer's extensive study of Devescovininae has shown that those genera are distinct from *Devescovina*. *Macrotrichomonas* Grassi is regarded as a synonym of *Gigantomonas* Dogiel by Connell (1932), but the validity of the distinction is clear to the writer after a study of specimens of both genera. Duboseq and Grassé (1925) believe that *Dinenympha* is a synonym of *Pyrsonympha*, but there still seem to be valid grounds for separation. Although Cleveland (1934, p. 281) is certain that *Proboscidiella* is a synonym of *Microrhopalocina*, Duboseq and Grassé's (1934a) description and conclu-

STRELOMASTIGIDAE.....	1+	5	3	0	0	0	0	0	0	0	0	0	0
Streblomastix.....	1+	5	3	0	0	0	0	0	0	0	0	0	0
PYRSONYMPHIDAE.....	38	55	11	1	0	0	0	0	0	0	6	0	0
SACCINOBAECULINAE.....	3	1	1	1	0	0	0	0	0	0	0	0	0
Saccinobaculus.....	3	1	1	1	0	0	0	0	0	0	0	0	0
PYRSONYMPHINAE.....	13	6	1	1	0	0	0	0	0	0	6	0	0
Pyrsonympha.....	5	6	1	0	0	0	0	0	0	0	6	0	0
Dinenympha.....	8	6	1	0	0	0	0	0	0	0	6	0	0
OXYMONADINAE.....	22—	48+	9	0	0	0	0	0	0	0	48+	0	0
Oxymonas.....	18—	43+	8	0	0	0	0	0	0	0	43+	0	0
Proboscidiella.....	2	2	2	0	0	0	0	0	0	0	2	0	0
Microrhopalodina.....	1	1	1	0	0	0	0	0	0	0	1	0	0
Kirbyella.....	1+	3	2	0	0	0	0	0	0	0	3	0	0
CHILOMASTIGIDAE.....	1	1	1	0	0	0	0	0	0	0	0	0	1
Chilomastix.....	1	1	1	0	0	0	0	0	0	0	0	0	1
HEXAMITIDAE.....	2	2	2	1	1	0	0	0	0	0	0	0	0
Hexamita.....	2	2	2	1	1	0	0	0	0	0	0	0	0
CALONYMPHIDAE.....	13	58?	7	0	0	0	0	1?	0	0	57	0	0
Coronympha.....	2	5	1	0	0	0	0	0	0	0	5	0	0
Metacoronympha.....	1+	4	1	0	0	0	0	0	0	0	4	0	0
Stephanonympha.....	5+	19+	5	0	0	0	0	0	0	0	19+	0	0
Calonympha.....	4+	15+	6	0	0	0	0	0	0	0	15+	0	0
Snyderella.....	1	1	1	0	0	0	0	0	0	0	1	0	0
"CALONYMPHID'".	—	21?	—	0	0	0	0	1?	0	0	20	0	0
HYPERMASTIGIDA.....	85	96	24	1	1	1	1	4	11	46	26	0	0
Lophomonadidae.....	3	2	2	0	0	0	0	0	0	1	0	0	0
Lophomonas.....	2	1	1	0	0	0	0	0	0	0	0	0	0
Kofoidia.....	1	1	1	0	0	0	0	0	0	1	0	0	0
JOENIIDAE.....	11	14	6	1	0	0	0	0	4	3	6	0	0
Microjoenia.....	4	7	2	0	0	0	0	0	1	0	6	0	0
Prolophomonas.....	1	1	1	1	0	0	0	0	0	0	0	0	0
Joenia.....	4?	5	3	0	0	0	0	2	0	3	0	0	0
Joenina.....	1	3	1	0	0	0	0	0	3	0	0	0	0
Joenopsis.....	1	1	1	0	0	0	0	0	1	0	0	0	0

TABLE 2—(Concluded)

Genera of flagellates	No. of spp.	Distribution of species											
		No. of hosts	Genera or subgenera of hosts	Number of species of hosts in which occur									
				Roaches		Hodo- termitidae	Kalotermitidae		Rhinotermitidae		Termitidae		
				Crypto- cercus	Cock- roaches		Lower	Kalo- termes	Reticuli- termes	Others			
155+T	26+T	1	1	1	4	11	105	6	26	About 26	0		
TOTAL	227+	2	2	1	0	0	0	0	1	0	0	0	
HOPLOTYPHIDAE	7	1	1	0	0	0	0	0	1	0	0	0	
Hoplonympha	1	1	1	0	0	0	0	0	1	0	0	0	
Urinympha	1	1	1	1	0	0	0	0	0	0	0	0	
Rhynchonympha	1	1	1	1	0	0	0	0	0	0	0	0	
Barbulanympha	4	1	1	1	0	0	0	0	0	0	0	0	
STAUROJOENINIDAE	3+	12	3	1	0	0	0	0	11	0	0	0	
Staurojoenia	2+	11	2	0	0	0	0	0	11	0	0	0	
Idionympha	1	1	1	1	0	0	0	0	0	0	0	0	
EUCOMONYMPHIDAE	8	27	8	1	1	0	1	0	0	0	26	0	
Eucomonympha	1	1	1	1	0	0	0	0	0	0	0	0	
Pseudotrichonympha	5	26	7	0	0	0	0	0	0	0	26	0	
Deltotrichonympha	1	1	1	0	0	0	1	0	0	0	0	0	
Mixotricha	1	1	1	0	0	0	1	0	0	0	0	0	
TRICHONYMPHIDAE	21	46	11	1	0	0	0	2	7	30	6	0	
Trichonympha	21	46	11	1	0	0	0	2	7	30	6	0	
CYCLONYMPHIDAE	1	1	1	0	0	0	0	0	0	0	0	0	
Cyclonympha (= Teratonympha)	1	1	1	0	0	0	0	0	0	0	1	0	
HOLOMASTIGOTIDAE	31	39	10	1	0	0	0	4	6	1	6	21	
Spirotrichonympha	11	27	9	0	0	0	0	3	3	1	6	14	
Spironympha (= Microspironympha)	3+	16	5+	0	0	0	0	4?	1	1	6	4++	
Spirotrichonymphella	1	2	1	0	0	0	0	0	2	0	0	0	
Holomastigotes	4	10	4	0	0	0	0	2	2	0	6	0	

HOLOMASTIGOTIDAE—(Continued)										
Holomastigotoides.....	5	22	7	0	0	0	2	0	0	0
Macrospironympha.....	1	1	1	1	0	0	0	0	0	20
Leptospironympha.....	3	1	1	1	0	0	0	0	0	0
Spirotrichosoma.....	3+	3	1	0	0	3	0	0	0	0
OTHER PROTOZOA										
Amoebae.....	10+	3+T	5+	0	1	0	2+	0	0	Many
Nyctotherus.....	3+	5+T	6+	0	1	1	2+	1	0	Many
Gregarines.....	3	5+T	5+	1	1	0	0	0	1+	Several
Other Sporozoa.....	6	6	6	1	1	0	0	0	1	2

sions indicate certain important differences, so that for the present they are left separate.

In considering the distribution of flagellates in termites, correctness of host determination is very important, and the nature of the faunas reported indicates that significant errors were made in the determination of a number of hosts. This might confuse a reader unfamiliar with all the facts. Dogiel (1917) described *Cyclonympha strobila* from *Coptotermes*, and Fedorowa (1923) recorded *Pyronympha* and *Dinenympha* also from Japanese *Coptotermes*. A comparison of their species with those described by Koidzumi from *Reticulitermes speratus* of Japan indicates that they actually worked with that termite. Mello and Brito (1929), in describing *Metastephanonympha* and certain species of *Devescovina*, considered that the host is a species of *Coptotermes*, but what is known of the Protozoa makes it certain that they were incorrect, and that actually it is one of the *Kalotermes* group. It would be almost as astonishing to find certain genera of termite flagellates in some termites as to find no Protozoa at all in a species of one of the four more primitive families.

In table 2 the first column of figures gives the number of species recognized in the genera and larger groups of flagellates. Often this is greater than the number that has been described, as it includes unpublished species. As many faunas of the termites examined, particularly those obtained recently in Africa and Madagascar, have not yet been studied in great detail, the number of species that will eventually be recognized in the writer's collections will be much greater than that here reported.

The second column of figures gives the number of host species in which flagellates of the genera listed have been found. Since the termites in the collections have not yet all been studied taxonomically, the record of number of species, and consequently the number of hosts in which the flagellates occur, is subject to some revision. The number of hosts probably will be increased over that given, rather than reduced.

In consideration of the Protozoa occurring in cockroaches, the number of hosts and genera of hosts have been given as one, although actually all these probably occur in three—*Blatta orientalis*, *Periplaneta americana*, and *Blattella germanica*.

The letter T. following the number under "number of hosts" or "genera or subgenera of hosts" means that the group under consideration also occurs in the Termitidae. Less effort has been made to give specific figures in that group than in the four lower families, because for the purposes of this paper the flagellates of the Termitidae are of little significance.

For the sake of economy, figures to illustrate the Protozoa considered in this paper are not included. Illustrations of most genera can be found by referring to Kirby (1934), Kudo (1931), Cleveland (1934), and Doflein (1927-1929). The description of the genus *Metacoronympha* was in press at the time of publication of this paper. It is included in the list because of its importance in the series of Calonymphidae.

SUMMARY

1. The writer has available for study the Protozoa of about a third of the termites of the four lower families, comprising about 430 species, all of which contain rich flagellate faunas; and has examined more than forty species of the fifth family, the Termitidae, which usually contain few or no Protozoa.

2. The problem of host-parasite specificity in the group is examined, and reasons are given for believing that below the Termitidae, at least, cross infection among species has not been an important factor in determining the present distribution of faunas. It seems that the flagellates have accompanied the termites during their phylogenetic development.

3. Various features of present distribution of flagellates in termites are discussed. It is pointed out that there is reason for believing that much of the evolutionary development of the flagellates took place in the primitive insects ancestral to roaches and termites; whereas some groups, of polymastigotes in particular, seem to have developed and diversified in termites.

4. The value of the entozoic faunas of the lower families in throwing light on the genetic relationships of termites is discussed and illustrated.

5. The Protozoa of the Termitidae do not have this value for correlative phylogenetic studies, but the presence of large amoebae in all examined species of the *Mirotermes-Cubitermes* group is of special interest. Such amoebae are not present in other termites so far studied, though most if not all species of *Amitermes* contain small ones.

6. A table is given listing the recognized genera of flagellates in termites and roaches, and giving the number of recognized species with their distribution in the larger groups of the insects.

It is not possible to summarize the facts presented in this paper, which is itself a summary treatment of the subject.

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THE DEVESCOVINID FLAGELLATE
PARAJOENIA GRASSII
FROM A HAWAIIAN TERMITE

BY
HAROLD KIRBY, JR.

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THE DEVESCOVINID FLAGELLATE PARAJOENIA GRASSII FROM A HAWAIIAN TERMITE

BY

HAROLD KIRBY, JR.

PARAJOENIA GRASSII was described by Janicki (1911, 1915) from a species of *Kaloterme*s from Hawaii. This termite is now known as *Kaloterme*s (*Neoterme*s) *connexus* Snyder, 1922; Janicki was incorrect in considering it to be *Kaloterme*s (*Neoterme*s) *castaneus* Burmeister. As described, the flagellate is of unusual interest in that it seems to be a transitional form between polymastigotes and hypermastigotes, and possesses a double parabasal apparatus. Through the kindness of D. T. Fullaway and of A. M. Adamson, the writer has been able to study living and stained material, with the result that important corrections of Janicki's account have been found necessary.

The genus *Parajoenia*, because of its supposed possession of many flagella on the anterior part of the body, was placed by Janicki (1915) in the family Joeniidae of the order Hypermastigina. Thus it was distantly removed from the genus *Devescovina* of the family Tetramitidae in the order Polymastigina, and associated with *Mesajoenia*, *Joenia*, and *Microjoenia*, genera which likewise possess an axostyle and have numerous flagella distributed on the anterior part of the body. The classification established by Janicki has been followed by many protozoölogists, including Doflein (1910), Kirby (1926*a*), Doflein-Reichenow (1928), Calkins (1926, 1933), and Kudo (1931). Dubosq and Grassé (1933) have accepted Janicki's classification and noted that in *Parajoenia* the characters of tetramitids and hypermastigotes are united.

Yet *Parajoenia* resembles the devescovinids in having a cresta, trailing flagellum, *Metadevescovina*-like nucleus, a devescovinid type of axostyle and not the *Joenia* type, and a parabasal apparatus which, though unusual, is of the devescovinid and not the *Joenia* type. It should at once occur to one familiar with devescovinids that the anterior flagellar apparatus should be reëxamined.

Light (1926) suggested that *Parajoenia* is in need of reëxamination in the light of our present-day knowledge of adherent bacteria. The writer (1930) listed *Parajoenia* in the family Devescovinidae, and (1931*a*) in the subfamily Devescovininae, but, for lack of space, gave no reason for so doing. In 1928, he had examined living material from *Neoterme*s *connexus* with the aid of dark-field illumination, and observed at once the usual three long anterior flagella of a devescovinid, which were overlooked by Janicki. The so-called anterior flagella described by Janicki are adherent spirochaetes.

The proper taxonomic position of *Parajoenia*, then, is among the Devescovininae. The speculations of Dogiel (1929) concerning the progressive polymerization of the anterior flagellar apparatus in the series *Devescovina*, *Metadevescovina*, and *Parajoenia* are completely invalidated, as the so-called ter-

tiary flagella of *Metadevescovina* are also adherent spirochaetes. In its type of nucleus, axostyle, cresta, and flagella, the organism shows a close relationship to *Metadevescovina*. Generic separation can be based only on the unusual parabasal apparatus of *Parajoenia*.

The body is stout (pl. 13, fig. 7), but is not smoothly ellipsoidal as shown by Janicki (fig. A). Janicki's measurements of about $43 \times 25\mu$ agree closely

with those reported in this paper. As in other devescovinids, the flagella arise in a small anteroventral depression. Anterior to this is a bluntly pointed papilla along which to its end the three long, delicate, anterior flagella are adherent. The trailing flagellum is a moderately stout cord, in length about $1\frac{1}{2}$ times the body length.

Janicki states that he was unable to demonstrate the blepharoplast clearly, but he described and figured it as comparatively large, spherical, with a distinct membranous boundary within which are several small, deeply staining granules (fig. A). The blepharoplast observed by the writer is by no means so large as that shown by Janicki. It is composed, as in many other devescovinids, of four or five closely aggregated granules (pl. 13, fig. 1).

The cresta (pl. 13, fig. 4) is in both shape and size very much like that of *Devescovina lemniscata* (Kirby, 1926b). It is longer than is indicated in Janicki's figures, in which, according to the stated magnification, its length is only 3.7μ . In

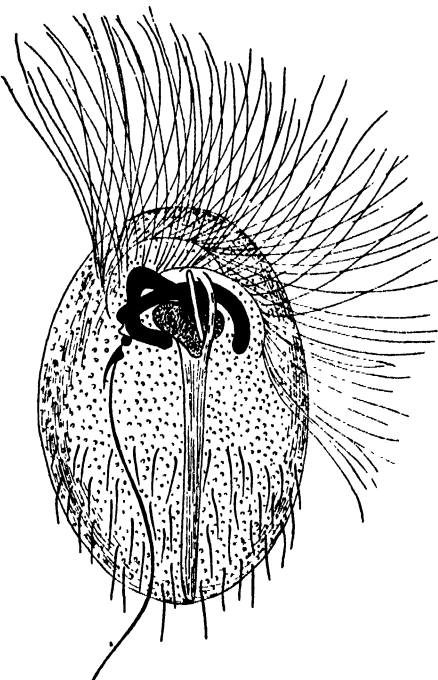


Fig. A. *Parajoenia grassii* according to Janicki (1915). After his plate 14, fig. 18. Corrections: the numerous supposed anterior flagella, and the posterior so-called bristle-like flagella, are adherent spirochaetes, and should not differ so much in size; the axostylar capitulum is not correctly shown; the cresta should be larger; the relation of the two elements of the parabasal apparatus to the blepharoplast is incorrect (cf. fig. 7, pl. 13). $\times 1500$.

the writer's material it has usually been found to vary from 7.5 to 11μ , in exceptional specimens (pl. 13, fig. 1) reaching 14μ .

Anteriorly the axostyle is expanded on the right side of the nucleus into a large capitulum which occupies a good part of the anterior region of the body, but does not give rise to a curved membranous expansion like that of *Metadevescovina debilis* and other species. From certain aspects (pl. 13, fig. 2) it often has somewhat the shape of a ginkgo leaf, the trunk being like a very much broadened petiole.

Janicki noted that the axostyle expanded behind the nucleus to receive a posterior protuberance of this, and gave origin to two narrow processes

which clasped the dorsal part of the nucleus on either side and joined each other anteriorly. In describing this arrangement, Janicki probably observed, but misinterpreted, certain parts of the axostylar capitulum.

Ventrally the capitulum extends to the region of the blepharoplast, far beyond the nucleus, which is at an unusual distance from the blepharoplast. The dorsal part of the capitulum extends about as far as the ventral part from the trunk, and anteriorly the capitulum reaches close to the anterior edge of the body. From the capitulum and the anterior part of the trunk, there rises, on the right side, a high, narrow ridge which extends anterior to the nucleus (pl. 13, figs. 2, 3). In it, or on it, there is a layer of abundant, small, chromatic granules. Janicki may have based his description of one of the anterior clasping processes of the axostyle on this structure, but his figures all show it in the wrong position on the left side of the nucleus. On the anterior part of the capitulum there are also small chromatic granules, grouped variously and often formed in a line along the anterodorsal edge.

The trunk of the axostyle is unusually stout. There is a membranous sheath, described by Janicki, bounding the trunk of the axostyle and the capitulum. This stains readily with iron haematoxylin. Within the sheath is an axial part which appears quite homogeneous and dark, and which is separated from the sheath by a clear space. In the interior of its anterior part, however, there sometimes appears a darker line. The axial part appears to terminate at the posterior end of the nucleus. Both sheath and axial part continue posteriorly to form the pointed, but not enlarged, cusp which projects from the posterior end of the body.

Janicki describes the axostyle as fibrillar, by which he apparently means that the part within the membranous sheath is fibrillar. The writer observed nothing like the numerous fine fibrils figured by Janicki. Janicki also states that the axostyle often projects a little beyond the body surface, but he figures it as completely enclosed and broadly rounded at the end.

The parabasal apparatus (pl. 13, figs. 4, 7) consists of two apparently separate parts. One of these, which may be termed proximal, is shaped like a V or an L. The bend, which often is a rather sharp right angle, sometimes a smaller angle, is situated close to the blepharoplast. The plane passing through the two limbs of this body is more or less in the longitudinal plane of the body. One limb extends anterior to the nucleus and the other posterior to this over the left side of the nucleus. The anterior limb usually is the shorter. The limbs may be curved, but no specimen has been observed in which, as Janicki shows in his plate 14, figure 18 (reproduced here as fig. A), the body had two sharp angles.

The second, distal part of the parabasal apparatus, which is comparable to the other in size, is curved, or even L-shaped; it also lies on the left side of the nucleus. The anterior end does not lie close to the blepharoplast, but close to or against the posterior part of the other portion of the apparatus, at a point nearer to its end than to the blepharoplast. The writer's observations on a large number of well-prepared specimens of *Parajoenia* are, in this respect also, not in agreement with Janicki's figure 18. His figure 19 de-

picts the relationship between the two elements of the parabasal apparatus more accurately.

The parabasal filaments can, as Janicki noted, be seen for the full length of both parts. In the proximal element, its bend is near the blepharoplast, and it seems to connect to that structure at this bend. At the anterior end, the filament extends freely for a short distance beyond the other substance of the parabasal (pl. 13, fig. 4). A branch of this filament passes from the posterior limb of the proximal parabasal element toward the blepharoplast, which it probably meets after paralleling the inner edge of the cresta for a short distance (pl. 13, figs. 4, 7). In the distal element the filament is particularly distinct, since it is, in part of its length, toward the posterior end, broadened to a narrow band which stains deeply. Narrowing to a filament again, it extends for some distance beyond the end of the other parabasal substance. At the anterior end, this filament connects the distal parabasal element to the proximal one, probably meeting the filament of the latter, though this point could not be determined with certainty. At the more or less sharp bend which usually characterizes the distal element anterior to its middle part, a short, curved branch filament is given off (pl. 13, fig. 1), clearly connecting with the main filament and extending free in the cytoplasm with no apparent function.

This description indicates that the parabasal apparatus of *Parajoenia grassii* is not really double, in the sense that there are two bodies connected independently to the blepharoplast, but that the second body is an appendage of the first, and is attached to the other part as are the cords of the parabasal apparatus of *Pseudodevescovina uniflagellata*, or the comparable "folioles" or parabasalialia of *Joenia annectens*.

In material stained in Delafield's haematoxylin after Schaudinn's fluid (pl. 13, fig. 5), the substance of the parabasal apparatus appears heterogeneous like that of many other devescovinids. Sometimes there is a series of stainable blocks alternating with clear areas, as in *Metadevescovina debilis* (Kirby, 1931b). Much more frequently, however, in the material examined, the stained ground substance of the parabasal contains clear vesicles. These may be arranged in a single row, scattered unevenly, or sometimes in parts of the bodies there are uneven, double rows of vesicles. A similar vacuolization has been observed in material stained in iron haematoxylin and counterstained in acid fuchsin after Schaudinn's fluid. The substance of the parabasal apparatus appears to be more labile than is usual in devescovinids. The outline is not so regular and in macerated material it seems to be destroyed more easily.

In a few specimens, the posterior ends of one or both portions of the parabasal apparatus were partly branched into two parts (pl. 13, figs. 1, 7), and in one the end of the distal portion was branched into several parts.

It is unnecessary to add anything to Janicki's accurate description of the form and structure of the nucleus. He describes a delicate membrane, finely divided chromatin filling the interior, and a rounded, usually peripheral nucleolus (Binnenkörper) in a clear area.

As a consequence of his fragmentary observations of division stages, Janicki

concluded that the parabasal apparatus probably is duplicated by division; that the chromatin undergoes division in finely granular form, forming no chromosomes; and that there is scarcely a doubt that the axostyle originates from the extranuclear spindle. This last point is not, however, supported by any illustrations.

The writer has observed very few division stages, and can present no conclusions concerning the mode of origin of the parabasal bodies. It would be of unusual interest to determine this point. Division of the parabasal body is most unlikely, however; that is not the method of duplication in other devescovinids. The figure of a telophase (pl. 13, fig. 8) shows clearly that, as in other trichomonads, the two new axostyles are differentiated without any relation to the paradesmose. The ends of the paradesmose are at a short distance from the blepharoplast, to which they are connected by fine filaments, as in other devescovinids. The parabasal bodies, although irregular in outline, are probably not double as Janicki supposed. In the specimen figured, there is associated with each body a long haematoxylin-staining filament, which probably is the filament of the distal part, the main substance of which has not yet developed.

Adherent spirochaetes, most of which are 15–20 μ long, are abundant on the anterior and posterior parts of the body (pl. 13, fig. 7), and the writer has seen no flagellates of this species without them. Those on the two parts are the same, despite Janicki's indication (fig. A) that they are very different. In some specimens the anterior spirochaetes may be lined up above the anterior edge of the capitulum. This may have given Janicki the impression of a row of flagella arising from a basal line. The granules often present on the capitulum might suggest basal granules. Most of the anterior spirochaetes are not associated with such underlying structures.

Janicki, who studied no living material, described bristle-like flagella on the posterior third of the body. Each "flagellum" was associated at its base with a rounded granule. Kudo (1931) and Duboscq and Grassé (1933) have already suggested that the so-called posterior flagella are adherent bacteria or spirochaetes, and the writer agrees with that interpretation. In living material, observed by dark-field illumination, and in osmic vapor-fixed material, stained with iron haematoxylin, they resemble the spirochaetes adherent to certain other flagellates in termites, and do not look like flagella or bristles. The rounded corpuscles described by Janicki, however, are associated with these filaments in a remarkable way. They are elongated, ellipsoidal, not circular as figured by Janicki, and one is present in the peripheral cytoplasm at the base of each filament (pl. 13, fig. 6). The filament seems to come into contact with the body surface above the corpuscle, at its posterior end or elsewhere, the point of apparent attachment not being constant. In the writer's preparations, filaments have been seen most clearly in Bouin-fixed iron haematoxylin material. Janicki believed that the staining reactions are like those of the parabasal body, but in the writer's material that is not so. The corpuscles are brownish and obscure in Flemming iron haematoxylin material, in which the parabasal body is black; they are unstained in Schaudinn-

Delafield material, in which the parabasal is stained like the chromatin of the nucleus; and they are not visible in osmic vapor-iron haematoxylin preparations, in which the parabasal is black. In the last preparations, the posterior spirochaetes are intensely stained, and indications are clear that the corpuscles are not a part of them. The nature of these cytoplasmic inclusions, and the reason for the remarkable association in position between them and the spirochaetes, remains to be determined.

Parajoenia Janicki

Parajoenia Janicki, 1911, Biol. Centralbl., 31:324; type species *P. grassii* Janicki.

Diagnosis.—A devescovicinid flagellate of moderately large size, rounded anteriorly and posteriorly, papilla not prominent; three anterior flagella; one long trailing flagellum; stout axostyle usually with pointed end projecting from the body, and expanded capitulum; parabasal apparatus of two parts, a proximal, V-shaped element attached at the apex to the blepharoplast and a distal element attached to the other part near its posterior end; nucleus with longer axis oblique or transverse, no clear space under membrane, chromatin granules small, one or more nucleoli present.

Parajoenia grassii Janicki

Parajoenia grassii Janicki, 1911, Biol. Centralbl., 31:324, fig. 3 [type host.—*K. (Neotermes) connexus* Snyder, Hawaii, incorrectly determined as *Calotermes castaneus* Burm.]. —Janicki, 1915, Zeitschr. wiss. Zool., 92:586, text figs. 3, 4, plate 14, figs. 18–22.

Diagnosis.—Dimensions of body: length 29–59 μ , averaging about 45 μ ; width 12–33 μ , averaging about 24 μ ; stout cordlike trailing flagellum about 0.3 μ maximum width, often 1½ times body length; cresta length 7.5–11 μ , exceptionally 14 μ , averaging about 9 μ ; two parts of parabasal apparatus of similar size, parabasal filament especially prominent in the distal part, in which it extends freely for some distance beyond the posterior end; short filament also extending into cytoplasm from posterior element of parabasal, at its bend near or anterior to the middle; trunk of axostyle stout, posterior end sharpened and projecting from cytoplasm, capitulum broad, with a high, narrow ridge or keel; nucleus 5–6.3 \times 2.3–3.4 μ , averaging 5.6 \times 3 μ ; numerous spirochaetes, about 15–20 μ long, adherent to anterior and posterior parts of body; those of posterior part attached over rounded, elongate, peripheral corpuscles.

SUMMARY

1. *Parajoenia grassii* Janicki, hitherto regarded as a hypermastigote flagellate, is a polymastigote of the subfamily Devescovicinae.

2. There are three long anterior flagella. The many anterior flagella described by Janicki are adherent spirochaetes.

3. The stout axostyle is expanded anteriorly in a broad capitulum, on the right side of the nucleus, and a high narrow ridge or keel rises from this capitulum. The axostyle is not fibrillar, as Janicki stated it to be.

4. There are not two parabasal structures, but one apparatus consisting of two elements. The proximal element is joined to the blepharoplast at a sharp bend near its middle point; the distal element is attached by its filament near the posterior end of the proximal element.

5. Janicki was incorrect in stating that the axostyles are formed from the spindle. The old one is resorbed and two new ones develop by outgrowth.

6. The spirochaetes adherent to the posterior part of the body are attached to the surface above peculiar ellipsoidal corpuscles in the peripheral cytoplasm.

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EXPLANATION OF PLATE 13

Abbreviations for methods of preparation: B., Bouin's fluid; Os. V., osmic vapor; S., Schaudinn's fluid, with 5 per cent acetic acid; D., Delafield's haematoxylin; H., Heidenhain's iron haematoxylin; Er., Erythrosin. Figs. 1, 7, and 8, $\times 1335$; others $\times 1830$.

PLATE 13

Parajoenia grassii Janicki from *Kalotermes* (*Neotermes*) *connexus* Snyder

Fig. 1. Axostyle, with pointed posterior projection, capitulum, projecting ridge with granular contents seen from left side; parabasal apparatus; flagella and unusually long cresta; adherent spirochaetes present on specimen, but omitted from figure. Fixed by D. T. Fullaway in Hawaii. Fixative not recorded, probably B. or S., stain H., Er.

Fig. 2. Axostylar capitulum, with ridge seen from its edge. Same slide as fig. 1.

Fig. 3. Capitulum from another aspect, ridge with granular contents on right; nucleus and parabasal. S., H.

Fig. 4. Semidiagrammatic figure showing anterior structures. Based on same specimen as fig. 7.

Fig. 5. Parabasal apparatus, showing heterogeneous structure. S., D.

Fig. 6. Rounded, elongated, peripheral corpuscles in posterior part of body, showing relationship to adherent spirochaetes. B., H. Fixed by A. M. Adamson in Hawaii.

Fig. 7. Typical figure, showing structure and adherent spirochaetes present on all specimens. Os. V., H.

Fig. 8. Division stage; new outgrowing axostyles; paradesmose; at each pole a single parabasal element, with irregular outline and long filament, attached near end, probably filament of distal element which is to develop later; new trailing flagellum more slender than old one. Same slide as figs. 1 and 2.



**THELAZIA CALIFORNIENSIS, A NEMATODE
EYE WORM OF DOG AND MAN, WITH
A REVIEW OF THE THELAZIAS
OF DOMESTIC ANIMALS**

BY

CHARLES A. KOFOID, OWEN L. WILLIAMS, AND N. C. VEALE

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CHARLES A. KOFOID, OWEN L. WILLIAMS, AND N. C. VEALE

IN AN EARLIER PAPER (1935) we reviewed a case of human thelaziasis from California. The parasite involved in that case, *Thelazia californiensis* Price, probably occurs only accidentally in man, but is a natural parasite of the dog in California. It is our purpose here to review briefly the thelazias parasitic in man and in the domestic mammals, to redescribe *Thelazia californiensis*, to give certain additional data with respect to the frequency of occurrence, distribution, and habits of the California thelazia, and to give a few notes on the diagnosis and treatment of thelaziasis of dogs.

HISTORICAL

Nematode worms of the genus *Thelazia* apparently were first discovered in domestic animals by J. B. Rhodes, who in 1818 removed a specimen from the eye of an ox in France. On the basis of Rhodes' brief description (unpublished), Bosc (1819) established the genus *Thelazia* (Thelazie), designating the species "Thelazie de Rhodes." Desmarests (1828) gave to this species the name *Thelazius rhodesii*, although he observed no specimens, and probably was actually concerned with an intraocular nematode of the genus *Setaria*. The original description of the thelazia of the ox was very inaccurate, but the host and location were given so that later workers were able to find and redescribe it. The best available descriptions of *Thelazia rhodesi* are those of Railliet and Henry (1910a) and Smit and Ihle (1931).

Railliet and Henry (1910a) have described two additional species of *Thelazia* from the ox, namely, *T. gulosa* and *T. alfortensis*. The second of these was described from female specimens only, and was separated from the first, *T. gulosa*, largely on the basis of the shape of the buccal capsule. Our observations indicate that the shape of the buccal capsule varies appreciably with the degree of contraction of the worm, and that this character is by no means a reliable one for use in the identification of species. We consider *T. gulosa* and *T. alfortensis* to be synonymous; the valid name becomes *T. gulosa*, the first of the two to be described in point of page position.

A third species of *Thelazia* parasitic in the eye of the ox is *T. skrjabini* Erschow (1928), a species quite distinct from the other two. It has been reported only from Russia

Thelazias have also been found rather commonly in the horse. Gurlt (1831) was the first to describe this species, naming it *Filaria lacrymalis*. Gurlt also obtained at least one thelazia from the ox, but failed to note that it differed from those of the horse. Railliet and Henry (1910b) reexamined Gurlt's original material and established the fact that the specimen from the ox was *T. gulosa*. Following Gurlt (1831), most parasitologists have referred to the thelazias of both ox and horse as *Filaria lacrymalis* (using several different spellings of the specific name), thus causing some confusion in the literature. This confusion was increased by the redescription of the equine thelazia under the name *Filaria palpebralis* by Wilson (1844). Following Wilson, a number of workers have used the latter name, an absolute synonym, for the thelazia of the horse, and the former name for the thelazia of the ox. Unfortunately, these errors are being perpetuated in twentieth-century texts (Law, 1903; Neumann, 1905; Law, 1909; Sharp, 1913; Neumann and Mayer, 1914; Edmonds and Walker, 1929; and probably others).

In connection with the taxonomy of the thelazias of the ox and the horse one further complicating factor should be mentioned. Smit and Noto-Soediro (1930) described a thelazia from the horse as *Thelazia floresiana*. From the original description this form was obviously very close to *T. rhodesi*, and Smit and Ihle (1931), after further study, pointed out that these two were synonymous. Thus *T. rhodesi* may occasionally be found in the horse as well as in the ox.

Summarizing, we find that the most common thelazia of the ox is *Thelazia rhodesi*, and that somewhat less commonly found in this host are *T. gulosa* and *T. skrjabini*. In the horse the thelazia of most frequent occurrence is *T. lacrymalis*, but *T. rhodesi* may also be found in this host. No reliable records of the presence of *T. lacrymalis* in the ox exist.

Leese (1910) found some thelazias in the eye of a camel in India. These specimens were sent to Railliet and Henry, who (1910a) described them as *Thelazia leesei*. This parasite is now known to be fairly common in camels.

Erschow (1928) found a thelazia in the eye of a pig in Russia, which he designated *Thelazia* sp. This same species was found in seven of three hundred and fifty swine examined by Oserskaja (1931). The latter investigator named the species *Thelazia erschowi*. Although this species has not yet been reported from countries other than Russia, it seems probable that it may eventually be found to have a wide distribution.

Finally, the two species of *Thelazia* parasitic in dogs and man remain to be mentioned. The first, *T. callipaeda*, was described by Railliet and Henry (1910b) from specimens collected from dogs in India, where it is reported to be a fairly common parasite. Later this species was found in dogs and also in man in China, indicating a wide distribution in Asia. *T. callipaeda* has once been reported as occurring naturally in a laboratory rabbit (Faust, 1928). Although Fischer (1919) and Faust (1927, 1928, 1929) have given some data on the morphology of *T. callipaeda*, the first full description of this nematode is that of Hsü (1933).

The second species of *Thelazia*, also found both in the dog and man, is *T. californiensis*. It was first reported by Allerton (1929) from a dog near Los Angeles, California. The specimens were identified by Dr. J. E. Ackert as *T. callipaeda*. Price (1930) received specimens of a thelazias from dogs in California, describing them as *T. californiensis*. Later, Price (1931) obtained additional material and redescribed the species. Dr. Ackert (*in litt.*) has re-examined the specimens in his possession and states that they agree with the descriptions of *T. californiensis*. Kofoid and Williams (1935) reported *T. californiensis* from dogs, and gave the first and only record of its occurrence in man.

Table 1 summarizes the available data on the occurrence and distribution of *Thelazia* in man and the domestic animals. Besides the species mentioned here, about fifteen others have been described from wild birds and mammals.

TABLE 1
OCCURRENCE AND DISTRIBUTION OF THELAZIA IN MAN AND DOMESTIC ANIMALS

Species	Host	Distribution	Authority
<i>T. rhodesi</i>	Ox	Europe	Bosc, 1819, etc.
		Africa (Egypt)	Railliet and Henry, 1910a
		Africa (Northern Rhodesia, Nyassaland, etc.)	Griffiths, 1922
		Asia (China)	Faust, 1927
		United States (California)	This paper
	Horse	Asia (East Indies)	Smit and Noto-Soediro, 1930
		(?) Asia (India)	Lingard, 1906
	Buffalo	Asia (Sumatra)	Railliet and Henry, 1910a
<i>T. gulosa</i>	Ox	Europe	Railliet and Henry, 1910a
<i>T. skrjabini</i> . .	Ox	Europe (Russia)	Erschow, 1928
<i>T. lacrymalis</i> .	Horse	Europe	Gurlt, 1831, etc.
		South America (Brazil)	Travassos, 1918
<i>T. leesei</i>	Camel	India	Railliet and Henry, 1910a, etc.
		(?) Africa	Edmonds and Walker, 1929
<i>T. erschowi</i> . .	Swine	Europe (Russia)	Oserakaja, 1931
<i>T. callipaeda</i> .	Dog	Asia (India)	Railliet and Henry, 1910b
		Asia (China)	Fischer, 1919, etc.
		Asia (China)	Stuckey, 1917, etc.
<i>T. californiensis</i>	Man	United States (California)	Allerton, 1929, etc.
	Dog	United States (California)	Kofoid and Williams, 1935
	Man	United States (California)	

THELAZIA CALIFORNIENSIS IN CALIFORNIA

Table 2 summarizes the cases of canine thelaziasis in California in which specimens of the parasites have been collected and positively identified.

Besides these known cases, there are reports of less definite character of eye worms of dogs in California, most of which probably refer to *Thelazia californiensis*. Price (1931) states that eye worms of dogs in California were reported verbally several years previous to Allerton's published record of 1929, but that no specimens of the parasites were saved.

Not long ago, in the neighborhood of Duarte, California, rumors were current to the effect that numerous cases of eye worms of dogs existed. We are not satisfied with respect to the reliability of these reports; if such infestations did occur, they cleared up before examinations could be made. Another report came to us from Berkeley, and from the description of worms and symptoms it seems probable that this was a case of thelaziasis.

Thelaziasis of dogs in California is probably more common than the available records of its occurrence would indicate. This opinion is based upon the fact that nine cases definitely referable to *Thelazia californiensis* have all ap-

TABLE 2
CANINE THELAZIASIS IN CALIFORNIA

Locality	Date	Eye infested	No. of worms	Authority
Los Angeles.....	1927	Both	12 or more	Allerton, 1929
Hollywood.....	1930	24	Price, 1931
Redding.....	1930	2	Price, 1931
Redding.....	1930	Price, 1931
Redding.....	1930	Price, 1931
Redding.....	1930	Price, 1931
Pasadena.....	1930	28	Price, 1931
Duarte.....	1932	Both	20	This paper
Calistoga.....	1932	Both	18	This paper

peared within a relatively short space of time. Furthermore, the infestation may easily be overlooked, and there is some evidence to indicate that the dog may in some way rid itself of the worms periodically, or that the worms are apparent only during a part of the year.

THE ADULT WORMS

The only available descriptions of *Thelazia californiensis* are those of Price (1930, 1931) and of Kofoid and Williams (1935). The latter description was based entirely upon specimens from man, which differed from those taken from dogs in that they were slightly smaller in nearly all measurements. The description given below is based upon specimens taken from dogs.

The worms are slender, tapering at the two ends. When alive they are whitish and translucent; fixed specimens are white, nearly opaque. The cuticula is annulated, the posterior borders of the annulations being sufficiently prominent to give the body a serrate appearance, especially in the anterior third. The annulations number from 32 to 83 per millimeter in the male worms, and from 35 to 66 per millimeter in the female worms. This character is of value in specific identification.

The head has a circle of eight papillae, which are grouped in pairs. Slightly posterior to these papillae is a pair of lateral papillae or amphids. We have not

been able to observe the inner circle of six oral papillae described by Price (1931) for this species, and similarly described by Hsü (1933) for *Thelazia callipaeda*. However, we observe six slight depressions in the cuticula surrounding the oral aperture which may correspond to the oral papillae described by the above-mentioned writers. These depressions were observed by Railliet and Henry (1910b) in their specimens of *T. callipaeda* from India.

The oral aperture is nearly round, and opens into a short, thick-walled buccal capsule which is somewhat greater in diameter than in depth. The shape

TABLE 3
MEASUREMENTS OF *THELAZIA CALIFORIENSIS*
(All measurements in millimeters)

Parts measured	Male	Female
Total length.....	7.7 -12.72	12.85 -18.80
Diameter.....	0.34 -0.50	0.36- 0.53
Head—diameter.....	0.066- 0.084	0.075- 0.090
Buccal capsule—length.....	0.021- 0.027	0.025- 0.030
Buccal capsule—diameter.....	0.027- 0.045	0.033- 0.051
Oesophagus—length.....	0.409- 0.541	0.554- 0.604
Oesophagus—diameter.....	0.057- 0.085	0.066- 0.088
Nerve ring from head end.....	0.252- 0.340	0.289- 0.327
Cervical papillae from anterior end.....	0.365- 0.460	0.353- 0.480
Left spicule—length.....	1.91 - 2.45
Left spicule—diameter.....	0.008- 0.014
Right spicule—length.....	0.160- 0.180
Right spicule—diameter.....	0.021- 0.036
Anus from posterior end.....	0.138- 0.163	0.100- 0.138
Vulva from anterior end.....	0.637- 0.945
Vulva from posterior end of oesophagus.....	0.105- 0.327

of the buccal capsule has been used by some writers as a diagnostic character. However, its shape varies so much with the degree of contraction of the specimen that it does not seem to be a reliable character for specific identification.

The buccal capsule opens into the triangular lumen of the oesophagus. The latter is moderate in length, and has its greatest diameter near its posterior end.

The vulva of the female opens to the outside at a point posterior to the posterior termination of the oesophagus. This character serves to distinguish the females of this species from those of *Thelazia callipaeda*, in which the vulva opens in front of the posterior end of the oesophagus.

The spicules of the male are dissimilar and very unequal, the left being from ten to fifteen times as long as the right. The right spicule has a maximum diameter two to three times that of the left. The preanal papillae number six or seven pairs; the postanal papillae number three or four pairs.

Table 3 gives the more important measurements of *Thelazia californiensis*.

LIFE-HISTORY STUDIES

Until the present time none of the efforts to discover the life history of the thelazias has met with success. In part this has been because of difficulty in obtaining infested dogs when needed, and in keeping the worms alive in the laboratory. Dogs in which the parasites have been discovered have been pets, and although loaned to us long enough to remove the worms, they were not available for experimental purposes.

Attempts to transplant the worms to other dogs were only partly successful; the worms did not survive more than a few days. Faust (1928) reports that some of the worms (*Thelazia callipaeda*) which he transplanted from one dog to another remained alive for as long as one year. He also had some success with the cat, the rabbit, and the monkey, although in each investigation there was noted a decrease in the number of worms, proving that they died out gradually.

In nature the dogs seem to acquire the parasites in late summer or early fall. Whether the parasites normally survive throughout the year is not known, but one occurrence of survival is worth mentioning. A pet dog in which the infestation was observed in August, 1932, was brought into the laboratory and the worms removed. The eyes cleared quickly and no further symptoms or worms appeared until August, 1933. Again the worms were removed. In the fall of 1934 the infestation once more appeared. Faust (1928) states that the occurrences of canine thelaziasis which he observed in China showed a similar seasonal distribution. The human infection described by Kofoid and Williams (1935) was detected in August.

Sheathed larvae are present in the uterus of female worms, and large numbers of these are forced out when the worms are placed in physiological salt solution. Cockroaches fed by us upon such larvae failed to become infected. Large numbers of larvae fed to dogs, or instilled directly into the conjunctival sac also failed to infect. Faust (1928) was unable to infect filth flies, the dog fly, and the cockroach with larvae of *Thelazia callipaeda*, nor was he able to infect dogs by direct ocular instillation with larvae.

None of the incomplete experiments made eliminates the possibility of an arthropod intermediate host with an infective larval stage, but no real clue on the method of transmission of this parasite has been uncovered. The portal of entry into the mammalian host is also problematical.

THELAZIASIS

The various species of *Thelazia* parasitic in mammals are approximately alike in their effects upon the host, such differences as do exist being mainly differences of degree of pathogenic activity rather than of kind. The worms live either in the lacrymal duct or the conjunctival sac. Frequently they can be seen to work their way actively across the surface of the eyeball, and again, to disappear into conjunctival sac or lacrymal duct. In one or two mammals,

thelazias have been reported as being present in the aqueous humor; if the identification of the parasites here involved was correct, the worms were obviously not in their normal situation.

In all mammals the thelazias cause an irritation of the conjunctiva which results eventually in a more or less severe conjunctivitis, with excessive lacrymation. In the known occurrences of canine thelaziasis the worms have been removed before extensive pathological changes occurred, although slight corneal opacity has been observed. The active movements of the worms apparently result in a gradual scarification of the cornea, with increasing opacity, and would very probably lead in time to complete blindness in dogs which were not properly treated. This is known to occur not infrequently among herds of cattle infested with *Thelazia rhodesi*, especially in Africa (Griffiths, 1922). In cattle, further and more severe pathological changes may follow, including secondary invasion by suppurative organisms which usually results in the complete destruction of the eyeball.

Diagnosis of thelaziasis depends upon a recognition of the primary symptoms—conjunctivitis and lacrymation—followed by the collection and determination of the worms. When many nematodes are present, they are not difficult to discover, but a careful search is required if the worms are few. Early recognition of the cause of the symptoms is important, as damage to the host's vision occurs in direct proportion to the length of time the parasites are present in the eye. However, observations on dogs, and more especially on cattle, indicate that no serious impairment of vision is likely to occur unless the infestation is allowed to remain untreated for a period of several months, or unless the number of worms present is abnormally large.

Treatment consists of removal of the worms from the eye. This is accomplished with forceps, or with a small cotton swab. Several subsequent examinations should be made, as it is not an easy matter to find all the worms at the first attempt, and less easy to catch and remove them, because of the rapidity of their movements. After-treatment should consist of an occasional washing of the eye with boric-acid solution until the inflammation has subsided.

SUMMARY

1. A brief history of the eight species of *Thelazia* parasitic in the domestic mammals and man is given.
2. *Thelazia alfortensis* is considered to be a synonym of *Thelazia gulosa*.
3. *Thelazia rhodesi* is reported from cattle in California.
4. The cases of canine thelaziasis in California are reviewed, and new cases reported.
5. *Thelazia californiensis* is redescribed.
6. The symptoms, diagnosis, and treatment of thelaziasis are outlined.

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THE ACTION OF SPECIFIC IMMUNE SERUM
AND OF NORMAL HUMAN SERUM ON INFECTIONS
OF *TRYPANOSOMA HIPPICUM* DARLING
IN THE RAT

BY

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INTRODUCTION

IN 1913 LAVERAN reported failure in efforts to immunize mice passively with serum from a rabbit which had been subjected to a series of inoculations of killed trypanosomes. His own investigation and a study of the literature led him to state that when the results of such efforts were not completely negative, they were at least inconsistent and partial. In concluding a section on the therapeutic and prophylactic action of immune sera, Taliaferro states (1929, p. 161) : "It seems to me there has been little accomplished to justify the hope of producing a serum definitely effective in the therapy of trypanosome infections." However, in view of the renewed investigations into the trypanocidal action of normal human serum, there is a growing interest in the immune reactions of specific trypanolytic sera. The present writer has therefore studied some phases of immunity to *Trypanosoma hippicum* Darling, and in spite of Laveran's failure (1913) to stimulate the production of an immune serum in a rabbit by the injection of killed trypanosomes, has again attacked the problem in the hope that such a serum might be obtained. While sera of high titre and polyvalent nature were produced, it was found (in confirmation of many earlier results) that although the life of the "protected" animal was prolonged, there was no general complete protection. However, certain results were obtained which seem to call for record and to invite further investigation.

To Dr. C. A. Kofoid and Dr. A. J. Salle the writer expresses his sincere gratitude for many helpful suggestions.

PROCEDURES

The strain.—A strain of *Trypanosoma hippicum* Darling was obtained in 1934 from the Hooper Foundation, and since then has been maintained in rats receiving heavy inoculating doses to avoid the development of relapse strains. Two rats were always used as passage animals, and when the trypanosome counts of one showed a marked decrement signifying a crisis, that rat was discarded. It is believed that in this way the strain was maintained in the original state.

Preparatory procedures.—Trypanosomes were isolated from rats for use as antigen in the preparation of specific antitrypanosome sera. Rabbits were given eight immunizing injections of this antigen and then bled out. The serum collected was used in attempts to immunize rats passively against the homologous strain of trypanosomes. In brief, for the completion of the problem it was necessary:

1. To free large numbers of trypanosomes of *all* the blood constituents of the rat host.

2. To use these isolated trypanosomes as antigen for the production of an immune serum in the rabbit; that is, actively immunize the rabbit by the injection of trypanosomes.

3. To immunize passively, and thereby protect, with the immune (antitrypanosome) serum thus produced, a susceptible animal, such as the rat, against the trypanosome.

It should be noted that the trypanosomes were isolated from the blood of the rat. Because the trypanolytic serum produced was to be tested on the same species, it was necessary to rid the trypanosome suspension used as antigen of every trace of rat blood. A trypanolytic serum made by the conventional methods was found to contain so much rat blood-cell material in the antigen as to be strongly antagonistic to rat blood cells, frequently killing the rats. The procedure described below was finally devised for isolating trypanosomes, since it had the advantage not only of giving a pure trypanosome suspension, but also of increasing the yield three- or fourfold over the older methods.

Trypanosomes are usually isolated by the use of fractional centrifugalization. With this method, by careful manipulation, it is possible to free some of the organisms of all the red cells of the blood. However, even with great care the organism cannot be freed entirely of the leucocytes unless well over three-fourths of the trypanosomes are sacrificed. Other workers have used hypotonic solutions (and even distilled water) to hemolyse the blood cells, but we found that the majority of the leucocytes, as well as the trypanosomes, withstood the swelling effect of the solutions. The efficiency of this method was not superior to that of repeated centrifugalizations, though less tedious.

The procedure developed for this work calls for a specific antirat hemolytic serum to remove the antigenic constituents of the infected blood. Because it is essential that even the leucocytes be removed from the trypanosome antigen, a sufficient quantity of leucocytes is included in the blood-cell antigen. For the production of this serum the following method is used:

A rat is anesthetized by ether and, with a single stroke of large shears, decapitated. The blood is collected in a petri dish containing 15 or 20 cc. of a 1.0 per cent sodium citrate-sodium chloride solution (Minchin and Thomson, 1915). This is transferred to a large (50-cc.) centrifuge tube and centrifuged for 20 minutes or more at 3000 r.p.m. to throw down all the white cells and as many of the platelets as possible. All of the buffy coat and the upper layer only of the red cells are pipetted off from the sedimented cells. These are re-suspended in Locke's solution (physiological saline-glucose) and seven or eight increasingly large doses of this suspension are given the rabbit intra-

venously at 4-day intervals. It is essential to include as many of the leucocytes as possible, in order to stimulate the production of amboceptors specific for them. In this method, a rabbit can be kept primarily for this purpose, the immune (hemolytic) serum withdrawn as needed, and the titre maintained by occasional injections of rat blood cells.

Preparation of the trypanosome antigen.—With a hemolytic serum produced as described above, it is possible to prepare a suspension of trypanosomes which is unusually free from all foreign proteins. It was found that the following procedure gives the best results in the isolation of the organisms.

Eight or ten days before the antigen is needed, two rats are infected with a heavy dose of trypanosomes. When the blood shows a heavy infection, the neck and thorax of the rat are thoroughly soaked with alcohol. The animal is then placed in a covered battery jar containing an amount of carbon dioxide sufficient to bring on a state of hyperactivity. While the rat displays marked hyperpnea, but before consciousness is lost, it is removed and, with large sterile shears, decapitated. The blood is collected in a petri dish containing about 15 cc. of the citrate solution. The use of CO₂ is not essential, but is desirable because as the CO₂ in the blood increases, reflexes in the animal cause venoconstriction with an emptying of the spleen, and higher blood pressure, and after the head is removed there is a greater tendency for the animal to have abdominal cramps. These reflexes increase the yield of blood and trypanosomes. For the effects of venoconstriction on trypanosome counts see the results of Poindexter (1933), who used phloeripine to cause contraction of the spleen.

Bacterial contamination is most likely to occur during decapitation and collection of the blood in the open petri dish. This can be avoided, of course, by bleeding from the heart, but the yield is very much lower by that method, and, as the chances of serious contamination are not grave with decapitation, this method has been used throughout the course of this work. Moreover, the antigen thus prepared has always been used immediately, thus reducing contamination. The citrated blood is then transferred to a 50-cc. centrifuge tube containing 20 cc. of warmed (37° C) Locke's solution, or, better, serum inactivated at 56° C for one-half hour. It is essential that the organisms be given either serum or Locke's, as sodium ions alone are toxic to the trypanosomes, owing, probably, to a lack of ion antagonism. To this suspension is added an excess of the hemolytic serum, 1.0 cc. being sufficient as a rule. The tube is kept in a water bath at 37° C for a few minutes, with frequent shaking. When clumps of the red cells can be seen macroscopically, the tube is placed in the centrifuge, which is run at a low speed (about 250 r.p.m.) for about five minutes. The supernatant portion, which should be of a pale pink tint, is then transferred by a sterile pipette to another tube and an excess of from 1.0 to 3.0 cc. of fresh guinea-pig complement is added. This causes lysis of the remaining erythrocytes as well as the white cells. A drop is removed and examined under the microscope; if red cells are seen, many of which are not in clumps, more hemolytic serum is added; if there are numerous clumps without signs of hemolysis, more complement must be added.

When dissolution seems complete, the trypanosome suspension is transferred to Hopkins vaccine tubes and centrifuged for 20 minutes or more at 3000 r.p.m. When the tubes are removed the trypanosomes will be seen sedimented at the bottom as a paper-white mass. Resuspension of the organisms should be carried out immediately. If they are to be used for agglutination titrations or for infecting inoculations, that is, if they are to be kept alive for more than a few hours, they should be suspended in the presence of inactivated serum (Yorke, Adams, and Murgatroyd, 1929). When the trypanosomes are to be used as antigen, that is, used in larger numbers and intravenously, it is wise to suspend them in Locke's in order to avoid the administration of so much foreign protein.

Preparation of antitrypanosome sera.—*Trypanosoma hippicum* runs a chronic course in the rabbit, with occasional crises and relapses. Death usually comes only after the infection has progressed for several weeks (Clark and Dunn, 1933). Consequently, it cannot be expected that the serum produced by rabbits during an infection would be of great potency; otherwise the infection would be terminated in short order. The experience of most workers has been that killed trypanosomes were only weakly antigenic, or that they were so changed that the antibodies produced were not specific for the living organism. Whether this change was caused by death *per se* or by disintegration of the organism following sometime after death, was unknown. There was also a question with respect to whether the living or the killed trypanosomes would produce a more efficacious antitrypanosome titre. In an attempt to answer these questions, two rabbits were given, semiweekly, eight heavy suspensions of living trypanosomes. Two other rabbits were given equal numbers of trypanosomes killed with 0.5 per cent phenol not more than 30 minutes previously, it being shown microscopically that disintegration did not progress far in that time.

Hereafter the immune serum from those rabbits receiving the living, active trypanosomes as antigen will be called "living-antigen serum," and the other serum, from rabbits receiving trypanosomes killed by phenol, "phenolized-antigen serum."

All four rabbits were given their inoculations from the same suspension and in equal doses. The first was given intraperitoneally; the remaining seven, slowly by the intravenous route. The doses ranged from 5×10^8 to 3.5×10^9 trypanosomes and were not given in increasing amounts, but were limited only by the amount available at the time, except that the final injection was the largest. Four days later the rabbits were bled out by cannulation of the carotids while the animals were under sodium amytol anesthesia. To the serum collected, phenol was added to a final concentration of 0.5 per cent. The sera were kept in the refrigerator, separately, being pooled only at the time of administration. Titrations were run on the sera from all four rabbits before and after immunization with the trypanosomes from the same passage strain, carried in the rat with heavy inoculating doses to avoid the introduction of relapse strains, and isolated by the same method. The results of these preliminary and other titrations are given below.

EXPERIMENTAL

Serum from each of the four rabbits to be immunized was tested before immunization was begun by microagglutination tests, an adaptation of the familiar Widal test. Except for a weak agglutination at 1:2 dilution in one serum only, the sera from these rabbits showed no specific agglutinins for the trypanosomes. Macroscopic trypanolytic tests were run on the different sera after immunization had been completed by the standard technic with a 2-hour observation period. The results are shown in table 1.

TABLE 1

IN VITRO TITRATIONS OF SERA OF RABBITS IMMUNIZED WITH KILLED OR WITH LIVING TRYPANOSOMES

Source of immune sera	Immune serum dilutions			
	1:100-1:2000	1:4000	1:8000	1:16,000
Phenolized-antigen serum. Rabbit No. 1	Lysis with few clumps	Most tryps. lysed, some sluggish	Many lysed, some free and fairly active	A few small clumps
Phenolized-antigen serum. Rabbit No. 2	Lysis with few clumps	Most tryps. lysed, few clumps, none active	Many lysed, very few moving	Several clumps
Living-antigen serum. Rabbit No. 3.	Lysis with few clumps	Most tryps. lysed, some sluggish	Many lysed, some free and fairly active	A few small clumps
Living-antigen serum. Rabbit No. 4	Lysis with few clumps	Most tryps. lysed, some sluggish	Many lysed, some free and fairly active	A few small clumps

NOTE: Control tubes showed no agglutination.

Several weeks later, when the *in vivo* experiments were completed, another series of titrations was run, but the results indicated very little, if any, deterioration in the titre.

Table 1 shows that the trypanolytic titres of the four sera are very nearly identical. This is a rather surprising result in view of the fact that the two rabbits given living organisms had antigenic trypanosomes in the blood throughout the course of the immunization period, whereas those given killed organisms had them only when administered. In spite of the massive doses of living, virulent trypanosomes repeatedly given these rabbits, the symptoms of the disease appeared only after a normal incubation period. There is perhaps only one plausible explanation for this, which, incidentally, explains the similarity between the trypanolytic titres.

It must be kept in mind that the same passage strain was given all these rabbits throughout immunization. Antibodies were produced in the rabbits

given killed trypanosomes only at the time of injection. Moreover, these antibodies were specific for only that particular strain. However, in the rabbits receiving the living organisms a typical host-parasite equilibrium was being sought with the first inoculation. The rabbit built up immune bodies against this initial strain, and in response the trypanosomes shifted to another, or relapse, strain. With subsequent inoculations, the new organisms were probably in large part killed before a relapse could take place. As a result, the response of the host to each successive inoculation was not different from that of rabbits receiving only the phenolized antigen. However, the relapse strains were continually changing, stimulating the production of antibodies specific

TABLE 2

IN VITRO TITRATIONS OF RELAPSE STRAINS OF TRYPANOSOMES IN RATS WITH SERA FROM RABBITS IMMUNIZED WITH LIVING TRYPANOSOMES

		Immune serum dilutions				
		1:100	1:1000	1:2000	1:4000	Control
Relapse strain A	Phenolized-antigen serum 1.....	++	—	—	—	—
	Phenolized-antigen serum 2.....	++	—	—	—	—
	Living-antigen serum 3.....	+++	++	+	—	—
	Living-antigen serum 4.....	+++	+	—	—	—
Relapse strain B	Phenolized-antigen serum 1.....	+	—	—	—	—
	Phenolized-antigen serum 2.....	+	—	—	—	—
	Living-antigen serum 3.....	+	—	—	—	—
	Living-antigen serum 4.....	++	++	+	—	—

for various strains. In this way, while the trypanolytic titre for the original strain was the same in all rabbits, those receiving the living antigen should have sera of a more polyvalent nature.

In the course of the work several titrations were run with these four sera on relapse strains which arose spontaneously in rats. In two of these titrations, in which were used strains A and B, shown in table 2, there is evidence which corroborates this explanation. The results show that although the relapse strain was only mildly agglutinated by the phenolized-antigen sera (probably a species-specific titre) the living-antigen sera (different ones in each titration) responded more vigorously.

Titrations run on several other heterologous strains showed only relatively weak, 1:1000 at most, immune reactions with the four sera, with only one exception, which was agglutinated by all the sera in dilutions comparable to the passage strain.

One may see from table 2 that the living-antigen sera are polyvalent, whereas the phenolized-antigen sera are not especially antagonistic to the strains tested. Because of this polyvalency one might conclude that these living-antigen sera are more efficacious when used with animals *in vivo*. This was not found to be true in the experiments described immediately below.

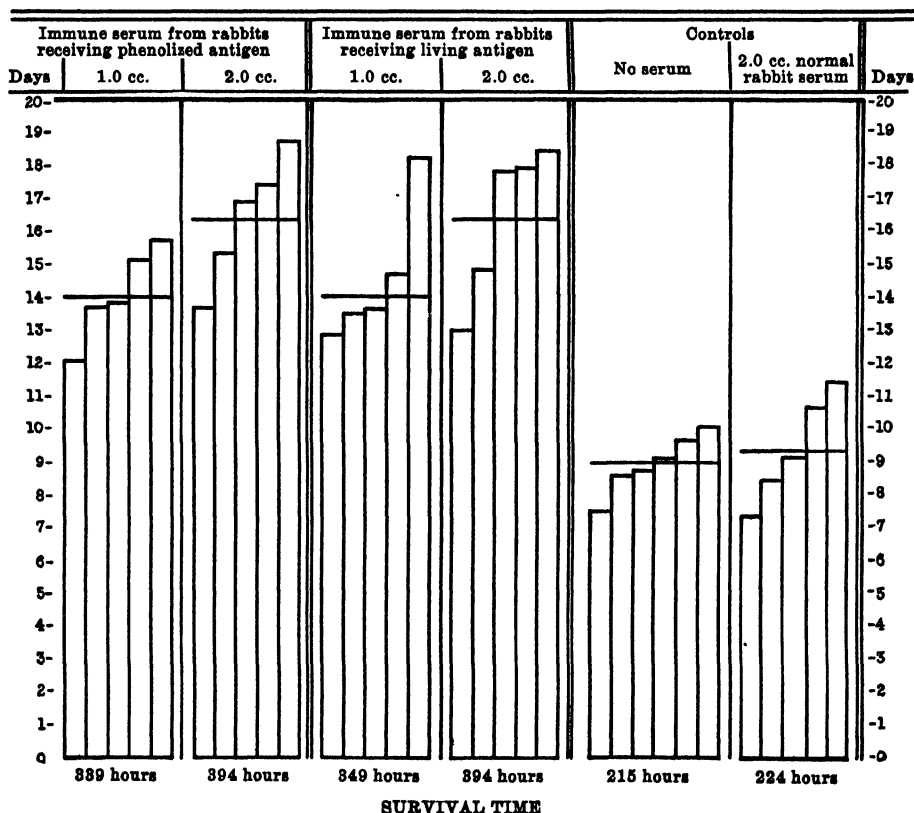
The protective action of the immune sera.—To test the protective value of the sera prepared, groups each of five healthy rats of 165–245 gm. weight, and averaging about 205 gm., were given intraperitoneally various amounts of the two immune sera, as follows :

- | | |
|--|--|
| Group 1—1.0 cc. phenolized-antigen serum | Group 4—2.0 cc. living-antigen serum |
| Group 2—2.0 cc. phenolized-antigen serum | Group 5—Control (6 rats), no serum |
| Group 3—1.0 cc. living-antigen serum | Group 6—Control, 2.0 cc. normal rabbit serum |

All rats were given in like manner 1,000,000 trypanosomes, calculated from counts in a counting chamber with Neubauer rulings. The results are shown graphically in table 3, which indicates that the two types of sera have

TABLE 3

SURVIVAL TIMES OF RATS RECEIVING 1.0- AND 2.0-CC. DOSES OF PHENOLIZED-ANTIGEN SERUM AND LIVING-ANTIGEN SERUM COMPARED WITH CONTROLS. THE INOCULATING DOSE WAS 1,000,000 TRYPANOSOMES. THE SERUM AND ORGANISMS WERE ADMINISTERED INTRAPERITONEALLY.



very similar protective qualities, and that the *in vivo* results are in strict accord with the *in vitro* titrations as shown in table 1. It may be supposed, therefore, that the active agents in the *in vitro* titrations of these sera on the

homologous strain are the same, or at least are developed to the same relative degree, as the immune bodies which bring about the protective action shown *in vivo*; that is, both of these reactions are probably to be ascribed to the same principle. In view of the similarity of the types of sera, the four sera were pooled in equal amounts in all subsequent work.

The fact that the two groups of rats receiving 2.0 cc. of immune sera outlived those receiving but 1.0 cc. led us to run further experiments using 3.0 and 4.0 cc. of the pooled sera from all four rabbits. Three rats were included in each group.

The inoculations were conducted as before. The controls lived, on an average, 223 hours, almost as long as the controls shown in table 3, indicating that comparisons may be drawn between the two groups although they were not run at the same time. A comparison of the average survival times of the two groups receiving 1.0 and 2.0 cc. of immune serum with the survival times of the rats receiving 3.0 and 4.0 cc. is shown in table 4.

TABLE 4

AVERAGE SURVIVAL TIME OF RATS RECEIVING INCREASING AMOUNTS OF IMMUNE SERUM COMPARED WITH CONTROLS. INOCULATING DOSE, 1,000,000 TRYPAOSOMES

Amount of immune serum administered	Number of rats tested	Average survival time in hours	Percentage of increase compared with controls
1.0 cc.....	10	344	156
2.0 cc.....	10	394	179
3.0 cc.....	3	455	207
4.0 cc.....	3	492	224
No serum (controls).....	13	219	100

Taliaferro and Johnson (1926) and Johnson (1929) have reported that the therapeutic value of immune serum was not dependent upon the size of the serum dose, but on whether or not the serum dose produced lysis. When the dose was sufficient to produce lysis in mice, the lives of the mice were prolonged about equally, irrespective of the dose size, but when no lysis occurred, there was no prolongation of life. In other words, the benefit of the serum lay in the production of a crisis, after which the infection progressed at a normal rate unhindered by the serum. The initial contact with the therapeutic serum causing lysis rendered the strain resistant, even when additional amounts of serum were administered. These results are somewhat in accord with those of the first studies by Franke (1905) and others, who found that trypanosomes can live in serum with high trypanolytic titres when the host has suffered from a relapse following a natural or an induced crisis.

A study of table 4 gives, however, an entirely different picture, for the survival times of the rats increase directly with increases in the amount of serum administered. Moreover, the effects of the larger amounts of serum were evident for some time even after trypanosomes appeared in the blood; that is,

there were numerous crises and relapses several days after the administration of the serum, which are only infrequently encountered with such large inoculating doses, even when serum in 1.0- or 2.0-cc. quantities is given simultaneously, as in table 3. This is a reaction which might be expected only when the serum exerts an inhibiting influence upon the trypanosomes even after the initially induced crisis. This, of course, is contrary to the findings of Franke and those confirming his results.

To check these results, three more groups of animals were run. One group received 2.0 cc. of the pooled serum and 1,500,000 trypanosomes, and 72 hours later a second dose of the same pooled serum was given. No trypanosomes were evident in the blood at this time. The second group received similar serum and infecting doses, but with an interval of 144 hours. There were trypanosomes present in the blood at the time of the second dose, although in small numbers. The third group received an initial dose of 2.0 cc. of phenolized-antigen serum and 1,500,000 trypanosomes. After 144 hours, 2.0 cc. of living-antigen serum was given. Trypanosomes were seen in the blood in this group also. Controls receiving no serum were run. The results are summarized in table 5.

Throughout the course of the infection in these rats there were also numerous crises and relapses, seeming to indicate that the effect of the serum was maintained for some time after the appearance of the supposedly resistant trypanosomes following administration of the serum.

Another interesting observation was made in groups 2 and 3, table 4, namely, that any trypanosomes present in the blood at the time of the second serum inoculation disappeared about 24 hours after the injection. This is conclusive evidence that the trypanosomes surviving the initial serum dose given 144 hours previously were still (or again?) susceptible, to some degree at least, to the action of that same serum. The incubation period following the disappearance of the trypanosomes was rather short, however, although when the trypanosomes did reappear they followed a very chronic course.

From a comparison of tables 4 and 5 it can be seen that the administration of serum in two doses is of greater therapeutic value than a dose of equal amount given at one time.

The action of normal human serum.—The prophylactic and therapeutic action of normal human serum on certain trypanosome infections of animals has been the subject of a vast amount of investigation. Laveran (1902) was the first to report the trypanolytic effect of normal human serum *in vivo*. Most of the trypanosomes infective for animals have been found since then to be susceptible to human serum. Laveran and Mesnil (1912) were of the opinion that human serum could not be studied *in vitro* because trypanosomes could be suspended in human serum with no evident injury. Rosenthal (1924) and his colleagues concluded that human serum contained no trypanocidal bodies, but was strongly trypanocidogenic; that is, the serum in itself was not injurious to the organisms, but when it was introduced into a host there was produced, in the destruction of the serum by the host, some product which was strongly trypanocidal. Yorke, Adams, and Murgatroyd (1930) announced that they had found (1929) that human serum *was* trypanocidal in its action

in vitro at 37° C. With *Trypanosoma equiperdum* they were able to destroy most of the organisms in four hours at dilutions of from 1:100 to 1:500. With *T. rhodesiense*, which is not so closely related to *T. hippicum*, the dilutions were higher, 1:5000 killing most of the organisms in four hours. Because of this action on the organisms *in vitro* the authors concluded that the therapeutic value of human sera has a "new significance," that is, it is not merely trypanocidogenic as Rosenthal (1924) and others have asserted, but has a direct action upon the organisms in contact with it.

Normal human serum was pooled by us from 10–15 Wassermann negative blood specimens sent into the California State Laboratories. Trypanolytic titrations with this normal human serum were run simultaneously with, and

TABLE 5

AVERAGE SURVIVAL TIME OF RATS RECEIVING IMMUNE SERUM IN TWO DOSES, AND OF CONTROLS. INOCULATING DOSE, 1,500,000 TRYPANOSOMES

Group	No. of rats	Initial dose of serum	Time interval (hrs.)	Second dose of serum	Survival time (hrs.)	Per cent as compared with controls
1	3	2.0 cc. pooled serum	72	2.0 cc. pooled serum	497	292
2	3	2.0 cc. pooled serum	144	2.0 cc. pooled serum	509	299
3	3*	2.0 cc. phenolized-antigen serum.....	144	2.0 cc. living-antigen serum.....	583*	343*
4	3	None.....	...	None.....	171	100

NOTE: Inoculating dose for all groups, 1.5×10^6 trypanosomes.

* One of the three rats died of an intercurrent infection and is not included in the averages. As the survival times of the other two rats showed appreciable difference (496 and 670 hours) the high average (583) cannot be considered necessarily significant.

under the same conditions as, the titrations with the specific immune sera listed in table 1. Although there was a definite immune reaction at 1:8000 (table 1) with those specific immune sera, the highest dilution at which there was any destruction of the organisms at the end of the two hours' observation period was at 1:64. Complete destruction took place at 1:32 and below. With a longer observation period of 18 hours, if the tubes were kept in an incubator at 37° C, partial destruction was observed at 1:256.

With this relatively low lysin titre it might be expected, from the recently reported results of Yorke *et al.* (1929, 1930), that the prophylactic (*in vivo*) action of the human serum would be correspondingly weak. These authors assert, as described above, that the action of the human serum is direct, that is, that the trypanocidal action *in vivo* is as easily demonstrable *in vitro*.

Normal human serum was given by us in 1.0- and 2.0-cc. quantities to two groups of three rats each. The inoculating dose was 1,000,000 trypanosomes, and the controls were those shown in table 3. None of the six treated rats became infected, although the controls died in about nine days. From this it can be seen that whereas 1.0 and 2.0 cc. of immune serum (with a 1:8000–1:16000 titre) merely prolonged the life of the rats for a few days, the same quantities

of normal human serum (with a titre of 1:64) gave complete protection to these few animals. More instances will be required to confirm these findings.

Yorke, Adams, and Murgatroyd (1929, 1930) may have demonstrated a direct lytic effect of human serum on trypanosomes in their *in vitro* work, but it can be seen from the results given here that this is not the only principle involved in these prophylactic doses of the serum. This discrepancy between the *in vitro* titres and the *in vivo* trypanocidal action of these two sera, we feel, gives weight to the older view of Rosenthal and others that the action of normal human serum *in vivo* is in large part indirect, and is not readily comparable to the action of specific trypanolytic sera.

In further confirmation of the theory that human serum is relatively ineffective *per se* but must be altered by the heterologous host in some way before becoming trypanocidal, are the results of Corson (1932) and Fairbairn (1933), who succeeded in infecting themselves with strains of trypanosomes which were apparently susceptible to the action of human serum *in vitro*. Adams (1933) and Culbertson (1934) also favor this view, stating that human serum sensitivity is not an indication of a lack of ability to infect man, and conversely, that immunity does not wholly depend on a trypanocidal serum, since their experimental baboon, with a low titre, proved resistant.

Thus we find that an immune serum with a fairly high titre which would normally be considered of therapeutic value is much less efficacious than normal human serum, which shows a very low titre *in vitro*.

SUMMARY

1. An improved method of separating trypanosomes from the blood constituents of the host is described. The technic calls for a potent hemolytic serum specific for all the blood cells of the host which is used for agglutination of the mass of the blood cells, followed by hemolysis of the remaining constituents.

2. The isolated trypanosomes were used as antigen in the production of two specific antitrypanosome sera. The production of one serum was stimulated by injecting rabbits with living trypanosomes; the other, with organisms killed by 0.5 per cent phenol.

3. Titres of both trypanolytic sera were virtually identical with regard to the strain used in immunization when tested *in vitro* (agglutination and lysis) and *in vivo* (protection).

4. The serum produced against living trypanosomes was of a more polyvalent nature (i.e., it was antagonistic to more strains) than the phenolized antigen serum when tested *in vitro* against several heterologous strains of the same species.

5. Protection experiments showed that the survival time of treated protected rats, contrary to the conclusions of Taliaferro and Johnson (1926) and Johnson (1929), was in direct proportion to the amount of protective immune serum administered.

6. Experiments showed that with periods of 72 and 144 hours elapsing between two doses of the same pooled sera, a crisis was induced after the second injection of serum. This seems to indicate that the trypanosomes were not completely resistant to the action of this serum even though they had survived former contact with it. This is in contrast to the results of Franke (1905) and many later investigators, who have reported that when trypanosomes once resist the action of a serum, they are resistant for numerous generations to further action of that same serum.

7. Pooled normal human serum was titrated under the same condition as the immune sera referred to above, and showed partial destruction of the trypanosomes at a dilution of only 1 : 64. *In vivo*, however, 1.0- and 2.0-cc. doses gave complete protection to rats whose controls died in nine days. Laveran and Mesnil (1912) and other early workers believed the definite *in vivo* action of normal human serum could not be demonstrated *in vitro*. Yorke, Adams, and Murgatroyd (1929, 1930) state that there is a direct action of the human serum on a suspension of the organisms *in vitro*. Several workers since then have shown that this direct action of the human serum does not protect even man himself from infection by a strain susceptible to the same human serum *in vitro*. The present work, by comparing specific immune sera of high titre with normal human serum of low *in vitro* titre, confirms the recent view that there is a slight *in vitro* action of human serum on the organisms, yet shows this is not responsible for the complete protection afforded rats when normal human serum is administered.

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CONTENTS

	PAGE
Introduction.....	249
Acknowledgments.....	249
Historical data.....	249
Materials and methods.....	250
Morphology.....	251
Orientation.....	251
General morphology.....	251
Habits and activities.....	254
The axial gradient.....	257
Ciliary wave patterns.....	258
The neuromotor system.....	260
Discussion.....	267
Summary.....	269
Literature cited.....	271
Explanation of plate.....	273

THE NEUROMOTOR SYSTEM OF NYCTOTHERUS HYLAE

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INTRODUCTION

FIBRILS have been observed in various species of *Nyctotherus*, but a coördinating function has never been ascribed to them (Maier, 1903; Bezzenberger, 1904; Daday, 1905; Ten Kate, 1927; Kirby, 1932). This paper shows that the fibrils of *N. hylae* are integrated in a motorium and that a neuromotor system is present. An analysis of the motor activities of the organism and the connections of the fibrils with the motor organelles lead to the conclusion that the neuromotor system has a coördinating function.

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HISTORICAL DATA

The species used in this investigation was described by Stein in 1862 and appears in his monograph on the Infusoria (1867) as *Nyctotherus cordiformis* var. *hylae*. To date thirty-five species have been described in *Nyctotherus*. These are listed with data on authors, hosts, and situation in the host, by Zelif (1933), who also described the last species, *N. osmodermæ*. *N. hylae*, heretofore known as a variety of *N. cordiformis*, is given specific standing in this paper. Table 1 supplements Zelif's (1933) list.

The description of *Nyctotherus cordiformis* var. *hylae* as given by Stein (1867), and a comparison of its structure with the diagnostic characters of other species of the genus, especially *N. cordiformis*, afford evidence sufficient to establish it as a distinct species on the basis of the relative sizes and shapes of *cordiformis* and *hylae*, host specificities, and the presence of the hyaline mass in *N. hylae*.

The fibrillar systems of the two forms present patterns sufficiently different to further warrant this conclusion. Ten Kate (1927) has given a detailed account of the fibrillar system of *N. cordiformis*, which the writer finds is not in agreement with that of *hylae*. There are many left-right fibrils in *cordiformis* which arise in the basal bodies of one side and pass through the endoplasm in a more or less direct manner to insert in the basal bodies of the opposite side. The endoplasm contains innumerable fibrils of this category. In *hylae* this same type of fibril is present but occurs in bundles (pl. 14, figs. 1-3, 6-7, 9) rather than separately as described by Ten Kate (1927) in *cordiformis*. It is

certain that many of the fibrils of the bundles arise separately from the base of the ectoplasm, probably from the basal bodies as in *cordiformis*. They converge into a bundle as they pass through the endoplasm, emerging separately as distinct fibrils as they reach the opposite side of the cytosome. There are two main bundles, one near the oral side and the other near the aboral border. Such left-right fibrils in *hylae* are more like those Ten Kate describes for *N. ovalis* than the ones he figures for *cordiformis*.

The caryophore fibrils in *hylae* surround the nucleus as in *cordiformis*, but the fibrillar nuclear envelope to which their inner ends are anchored in *hylae*

TABLE 1
NEW SPECIES OF *Nyctotherus*
A supplement to Zelif's table (1933)

Species	Host	Situation
<i>ampullarium</i> Cordero	<i>Ampullaria canaliculata</i> , <i>insularium</i> , <i>megastoma</i>	Intestine
<i>hylae</i> Stein	<i>Hyla regilla</i>	Rectum
<i>mozzai</i> Jorg		
<i>nankingensis</i> Nie	<i>Rana limnocharis</i>	Intestine
<i>pyriformis</i> Nie	<i>Rana limnocharis</i>	Intestine
<i>silvestrianus</i> Kirby	<i>Amihermes silvestrianus</i> , <i>minimus</i> , <i>emersoni</i>	Intestine

has never been found in *cordiformis*. Other minor structural features at variance in the fibrillar systems of *cordiformis* and *hylae* may be attributable to divergent interpretations by Ten Kate and myself.

Conchophthirius is a trichostomatous ciliate, but has many affinities with the heterotrich *Nyctotherus*. Membranelles are absent, but a well-developed pharynx and peristome are present. The fibrillar system described by Kidder (1934) for *Conchophthirius* is much like that to be described in this paper. Lucas (1934) has described a neuromotor system for *Metopus circumlabens*, a heterotrichous ciliate closely related to *Nyctotherus hylae* in some details of its neuromotor apparatus. In the opinion of Miss Lucas, the neuromotor system bears its more important relationships to the digestive, absorptive, and excretory organelles in the cytoplasmic body of the protozoan and especially to the ingestory cilia.

MATERIALS AND METHODS

Nyctotherus hylae used in this investigation were obtained from the rectal contents of the common tree frog, *Hyla regilla*. Frogs from various localities in the San Francisco Bay region have been found to harbor these commensals in great abundance, the incidence being about 80 per cent. For vital studies, the rectal contents were mixed with 0.85 per cent saline solution corrected to the pH (7.64) of the rectal contents. The material was then subdivided on vaseline-ringed slides. After the second or third day, the movements of *Nyctotherus* are slowed down and the opportunity for observation and analysis of movements greatly enhanced. Dark-field illumination of living material was

valuable in confirming many phenomena observed with ordinary light. Many phases of the work were aided by interpretations of slow-motion cinematographic films, both by the observation of the film, frame by frame, and by repeated projection. Both whole mounts and sections have been used. Whole mounts were fixed in Schaudinn's fluid with 5 per cent acetic acid and with Flemming's solution, and stained with Heidenhain's iron haematoxylin, aqueous and alcoholic. The silver techniques produced only negative results. The silver methods of Klein (1926), Gelei and Horváth (1931), and the Yabroff modification (1928) were all tried repeatedly but without results. The only silver preparations obtained were those in which the organisms were previously subjected to tap water for a short period. *Nyctotherus* will remain alive for several hours in tap water, which removes the salts commonly present when the animal is freshly taken from the rectum. Such preparations do not cause precipitation of the silver nitrate, and they do take the silver impregnation sufficiently to show up the ciliary basal bodies and their longitudinal connections. Organisms fresh from the rectal contents allowed to dry down on the slide and subsequently washed before subjection to the silver bath will not impregnate. Material removed from the rectum before fixation did not prove suitable for sectioning. All whole mounts were made from material which had been fixed extrarectally. Work with Chamber's micromanipulator has proved of no value, since the organisms are equipped with a heavy pellicle which is practically impenetrable to the quartz needles.

MORPHOLOGY

ORIENTATION

For the sake of clarity, and because of the decided asymmetry of the organism, an enumeration of the regional adjectives employed in this paper will be given. The region in which the cytostome is situated will be termed the "oral" region, and the opposite border the "aboral" region. The prominent lateral suture in the ciliary pattern is on the "left" side of the body. "Presutural" will refer to that part which lies between the lateral suture and the oral border. That region from the lateral suture to the aboral border is the "postsutural" region. The anal end of the body is "posterior," whereas the opposite end is "apical" or "anterior." That side of the body which functions as the ventral side is, in reality, the "right" side.

GENERAL MORPHOLOGY

The largest individuals of *Nyctotherus hylae* observed by Stein (1867) were 230μ in length. The organisms used in this investigation have been found to range from 168 by 90μ to 248 by 144μ (measured on fixed material). One exceptionally large individual measured in the living condition was 335 by 180μ .

The body of *N. hylae* is subject to appreciable variation in shape dependent on whether the organism is performing rotating or skating movements. When the organism is engaged in the usual nonrotating movements the body is flattened on the right side and somewhat convex on the left. In rotation one side of

the body is highly concave, and the other highly convex. The peristome, averaging 90μ in length, extends from near the apical end of the body on the left side of the oral border to the cytostome. The cytopharynx, a direct continuation of the peristome, has an average length of 110μ . It extends aborally from the cytostome in a transverse position (sometimes a little posteriorly) and then bends posteriorly in the form of a rounded right angle. The descending part of the pharynx is shorter than the transverse part. The adoral membranelle zone with approximately 100 membranelles extends from the apical end of the peristome to the inner end of the pharynx. At the inner end of the cytopharynx as it opens into the endoplasm there are three terminal membranelles which beat out of rhythm with those of the cytopharynx.

A short oral bristle (fig. C, *cyt. sh.*; pl. 14, fig. 1) extends from the floor of the cytostome. Stein (1867) asserts that it helps to direct food into the mouth. Daday (1905) describes a similar structure in *N. piscicola*. The writer finds that a very delicate membrane extends the full length of the bristle over to the right wall of the cytostome. The membrane is observable in living specimens only and then with difficulty, as the bristle and membrane are easily hidden by the active beating of the membranelles. The bristle has been seen in a few fixed specimens only, but is constantly present in living organisms. The bristle and membrane have never been seen to perform movements per se. This structure (bristle and membrane) is here named the "cytostomal shelf." The shelf definitely has been seen to direct food into the pharynx, an assumption earlier made by Stein (1867).

The body is uniformly covered with cilia closely set in longitudinal rows. Two sutures are present, a lateral one on the left side of the body (fig. A, 1-2; fig. C; *lat. sut.*), and the sagittal suture (fig. C, *sag. sut.*) extending posteriorly from the cytostome along the oral border, probably to the anus. The ciliation is divided into three morphological areas which function as two. These areas are: the presutural cilia, postsutural cilia, and right-side cilia, the two last-named functioning as one area. The *presutural cilia* are those on the left side of the body between the oral border and the lateral suture. The *postsutural cilia* are those between the lateral suture and the aboral border. The postsutural ciliary rows, which arch with the aboral border, have more curvature than the presutural ciliary rows, which arch with the oral border. The *right-side ciliary rows* all arise in the anterior end of the body at the oral border and lie parallel to the aboral border, terminating at the posterior end of the organism.

The macronucleus (fig. C, *mac.*; pl. 14, figs. 1-3, 6) is an asymmetrical bilobed structure, the aboral lobe being the larger, averaging 68μ in the longest axis and about 25μ wide in the narrowest region. The micronucleus is a small oval body situated in a concavity on the right side of the macronucleus in the median-posterior region. The nuclei are both surrounded by the nuclear envelope (fig. C., *nuc. env.*). Just anterior to the macronucleus is the large triangular hyaline mass (fig. C., *hyal. mass*; pl. 14, figs. 1-3, 6). Horning (1927) suggests that it is an aggregation of mitochondria.

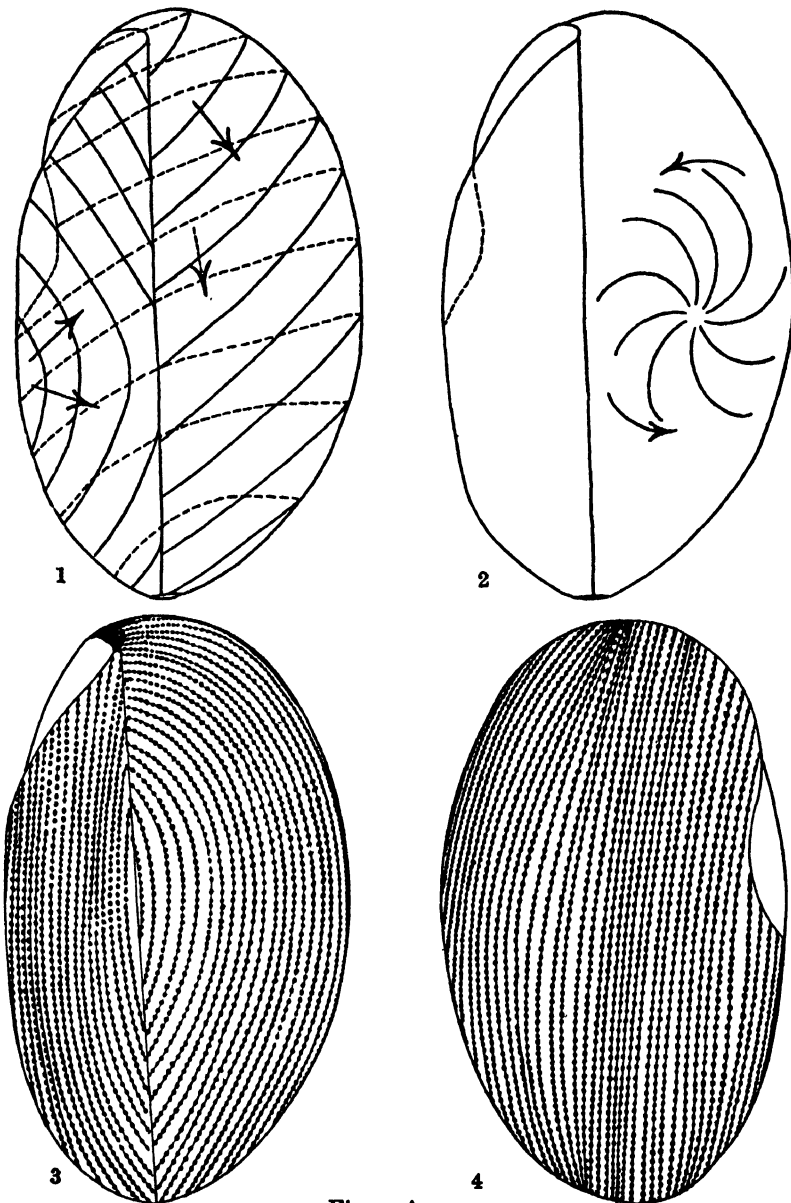


Figure A

Fig. A. $\times 335$. (1) Semidiagrammatic figure of the normal ciliary wave pattern in *Nyctotherus hylae*. The arrows indicate the direction of wave progression. The waves of the left side of the body are indicated by solid lines, those of the right side, by broken lines. (2) The vortical arrangement of ciliary waves in the aboral region at the onset of ciliary reversal. (3) The ciliary lines, basal bodies, commissural fibrils in the anterior region and the lateral suture, as seen from the left side. (4) The right side of the body showing ciliary lines and ciliary basal bodies.

Maier (1903) described a structure in *Nyctotherus cordiformis* which he termed the "Basalwulst" and to which he ascribed a supporting function. This Basalwulst, to which are rooted the membranelles of the peristomal and cytopharyngeal regions, appears to be a support for these vibrating organs. The Basalwulst is a broad structure extending throughout the length of the peristome and on the anterior side of the cytopharynx. At the posterior end of the pharynx it curves to the left over the pharynx and down under it, where it again turns and proceeds orally as a rodlike structure, tapering to a point and ending at the cytostome. This Basalwulst is visible in the living organism as a hyaline structure with several spherical refractile bodies which appear in the fixed specimens as vacuoles. It is quite likely that support is the sole function of this heavy ectoplasmic structure, since the cytopharynx retains its contour under all circumstances. In this paper the term "pharyngeal ectoplasmic thickening" will be applied to this structure (fig. D, 1; fig. E, *phar. ecto. thick.*, and *post. arm ecto. thick.*).

The contractile vacuole, which is formed by the coalescence of small vacuoles which gather about it, pulsates at intervals of about two minutes and thirty seconds. It lies in the posterior part of the body near the oral border (fig. C, *con. vac.*; pl. 14, figs. 2-3), communicating with the anus by means of a ciliated canal averaging 25μ in length. Higgins (1929) reported for *N. cordiformis* the presence of a second canal near the first, also communicating with the anal opening. It is not present in *N. cordiformis*, *ovalis*, *hylae*, or *velox* (?) observed by the author. The anal canal is not a cylindrical tube as has been supposed, but is a broad communicating passage flattened right to left. Into this large passage the pulsating vacuole empties its liquid contents. Solid wastes are also liberated into this canal, but not by way of the pulsating vacuole. The writer has not been able to establish a time interval for the liberation of solid wastes. They are evacuated with less regularity and in a more inconspicuous manner than are the liquid wastes.

HABITS AND ACTIVITIES

Nyctotherus feeds chiefly on the bacterial flora of the rectal tract. Ingestion of intestinal flagellates and red blood corpuscles has also been observed, and occasionally an organism will be seen gorged with them. However, flagellate feeding is probably only casual. Living flagellates have been seen to emerge from the anal aperture and resume their normal mode of activity unchanged by the transit; or they may lodge on the cytosomal shelf or in the cytopharynx without proceeding farther. *Nyctotherus* will feed voraciously on amphibian blood cells, which are ingested without any apparent difficulty and digested within twenty-four hours.

The membranelles of the peristome (pl. 14, figs. 3-4, 6) drive the food organisms to the region of the cytostomal shelf. The pharyngeal membranelles (figs. D, 1; E, *phar. memb.*; pl. 14, figs. 1-3, 5, 7-9) carry them to the inner end of the pharynx, where the terminal membranelles (fig. E, *term. memb.*; pl. 14, fig. 2) direct them into the endoplasm. A few of the ciliates were placed

in saline solution containing thousands of bacteria cultured from a smear of the rectum of the host. The bacteria were so numerous that one could see clearly the path they took from the apex of the body to the cytostome. Because the peristomal membranelles beat unceasingly, a stream of bacteria was contin-

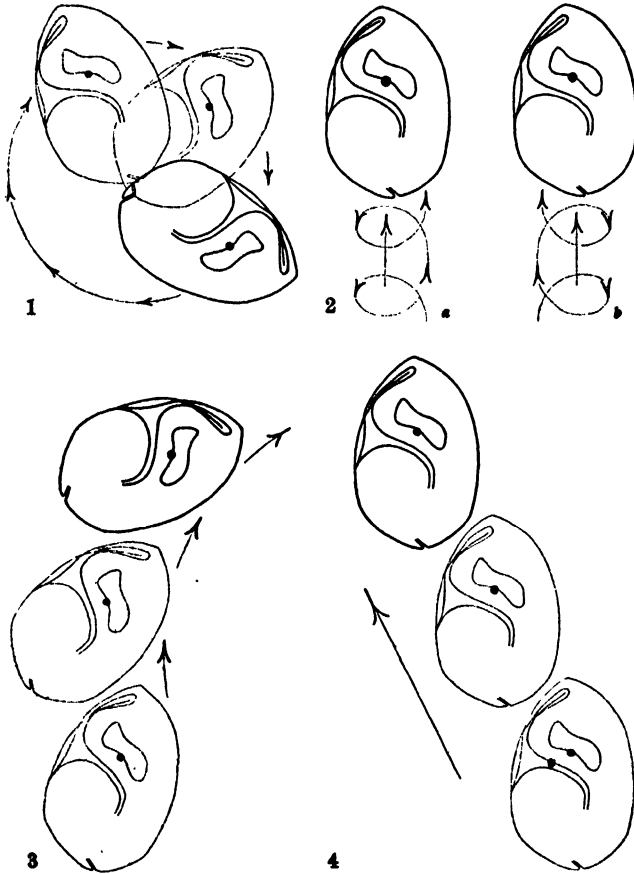


Figure B

Fig. B. Diagrammatic representations of the movements of *Nyctotherus hylae*, ca. $\times 110$. (1) Rapid aboral circling (spinning). (2a) Counterclockwise rotation. (2b) Clockwise rotation. (3) Slow aboral circling. (4) Forward progression without rotation as exhibited when the organism has one side applied to the substrate (skating).

uously being disgorged from the cytostome just posterior and to the left of the shelf, indicating that the food supply was greater than could be received.

From the cytopharynx the bacteria pass posteriorly and aborally into the cytoplasm. Definite large food vacuoles, such as are easily seen in many ciliates, are not apparent in this organism. Thus the course taken by the food is obscured and has never been described.

Nyctotherus is positively thigmotropic. When first removed from the intestine and placed in saline solution, the organisms exhibit rotating movements

as they swim. After an hour one finds that most of the organisms are swimming without rotation; they have settled to the bottom and are skating along on the substrate. They also exhibit a positive geotropic response, as shown by the fact that they generally swim with the left side uppermost and the right side applied to the substrate. In the slides of living material an individual is occasionally seen swimming with its right side uppermost instead of the left. By careful focusing it was found that such individuals were swimming, or skating, on the undersurface of the cover slip with the right side applied to the glass. Individuals swimming between the cover slip and the upper surface of the slide exhibit the rotation as they progress. Skating is far more common than rotation, and skating occurred most frequently on the surface of the slide rather than on the undersurface of the cover slip, an example of the geotropic, or equilibrium, response.

In the forward movement without rotation (fig. B, 4) the organism moves at an angle of 40 degrees to the left of the main axis. In a second type of skating movement the organism describes a large or small circle aborally (fig. B, 1 and 3). The large circle is the result of slow movement; the small orbit, of rapid action. The two rates involve two distinct ciliary wave patterns, to be discussed later.

In the rotatory movement (fig. B, 2, *a-b*), the animal normally moves directly anteriorly, rotating clockwise on its anteroposterior axis, scooping as it progresses. Deflections to the right or left in rotation have not been seen. In this rotation, the left side of the body is strongly convex and the right is deeply concave, giving a scooplike effect. The peristome is in its normal position, a little to the left of the oral border. The organism is capable of reversing both the direction and the "scoop" to swim in a counterclockwise fashion. When this occurs the convexity of the left side is reversed to the right side and, likewise, the concavity of the right side is reversed to the left; thus the organism is again scoop-shaped. The peristome has also become somewhat shifted in its relations, now lying much nearer to the oral border. Organisms swimming in a clockwise manner are seen to stop, shift into the reverse, and continue swimming, but in the counterclockwise manner. The change takes place so rapidly as to be almost imperceptible. The clockwise rotation is the more common.

Reversal of rotation is probably typical of all species of *Nyctotherus*. The author has witnessed this peculiar behavior in *N. cordiformis*, *N. ovalis*, *N. hylae*, and *N. velox* (?). Bragg (1934) has shown that reversal of rotation occurs in *Paramecium trichium*. The counterclockwise rotation of that species has always been one of its chief diagnostic characters. Bragg found that the organism occasionally exhibits clockwise rotation. This, however, is the exception rather than the rule, for it never persists long before reversing to counterclockwise rotation. This is the same phenomenon as that in *Nyctotherus*, in which, however, rotation is mainly clockwise, though occasionally the organism reverses its form to counterclockwise rotation, for a short period only. The writer has also seen reversal in *Paramecium multimicronucleata* and *P. caudatum*. In these forms, clockwise rotation is the rule, but periods of counter-

clockwise rotation occur. Further investigation will doubtless show that reversal of rotation may be a far more common phenomenon in other ciliates than has been previously supposed.

THE AXIAL GRADIENT

Child (1914) found that an axial gradient in susceptibility to KCN is present in *Stentor*, *Stylonychia*, *Vorticella*, *Carchesium*, and *Paramecium*. In these forms the anterior or apical region was found to be the highest point in the general gradient, although localized regions of still higher rate, such as vacuolar regions of *Paramecium*, were detected. He was able to demonstrate the axial gradient with a concentration of 0.002 M KCN, approximately 0.01 per cent. Bush (1934), working with *Haptophrya michiganensis*, found that concentrations approximating 0.1 per cent were necessary to demonstrate the gradient in that form. It has been found that *Nyctotherus hylae*, likewise, requires the higher concentrations. In 0.2 per cent KCN, the organisms circled around and around aborally, as in figure B, 1, but soon became acclimatized and resumed normal activity. A solution of 0.3 per cent was strong enough to demonstrate the gradient in an average of ten minutes. When placed in this stronger concentration of KCN, the organisms exhibit the aboral circling, which continues rapidly until the ciliary movement loses coördination. The animal then remains quiescent with the cilia beating almost synchronously over the entire body, producing a rhythmical jerking, as though the animal were entangled and trying to free itself. By the time this condition has been reached, the pharyngeal membranelles have ceased to function, although the entire group of peristomal membranelles remain active. This is probably the result of actual destruction of the motor elements in the pharynx, because absorption of the toxic agent into the endoplasm would occur here first. The cilia at the apex of the organism having soon come to rest, cessation of ciliary action progresses down the aboral region. All this time the cilia in the regions posterior to the cytostome and along the posterior part of the oral border retain rapid rhythmical activity. As soon as ciliary cessation moves down the aboral regions, the peristomal membranelles begin to beat more and more slowly until a break occurs at the apex and the endoplasm flows out rapidly, leaving the ectoplasm transparent and intact. All ciliary and membranelar activity then ceases. Often secondary eruptions occur in the peristomal region and in the apical-aboral region. At no time was disintegration observed in the cytopharynx so that the endoplasm exuded, although this region was the first to be affected when the organism was placed in the toxic solution. Individuals placed in 20 per cent alcohol exhibited the same general series of disintegratory phenomena.

It would therefore appear that *N. hylae* has a differential metabolic rate in its various parts, the highest being in the region of the apex, the lowest, in the area between the cytostome and the anus. It is also apparent from these results that the motor activities of organelles of the pellicle persist after the organism is moribund. Such activities, however, are noncoördinated.

CILINARY WAVE PATTERNS

The ciliary wave patterns of *Nyctotherus hylae* are definite and characteristic (fig. A, 1-2) but not directly correlated with the structural pattern of ciliary rows (fig. A, 3-4). The waves are clearly seen on the living organism and are easily recorded on the cinematograph film exposed at sped-up intervals. Subjected to dark-field illumination, each wave stands out as a distinct white line. However, little is seen by the dark-field method which is not made visible by ordinary illumination. Normally the waves arise from three centers, namely, the posterior or distal end of the cytopharynx, the posterior margin of the cytostome, and the apical end of the peristome.

At the *posterior end of the cytopharynx* the most pronounced or membranellar waves arise. A stimulus arising at this point progresses toward the cytostome, and to the apex of the body, causing a single membranellar wave to progress along the entire adoral zone. The waves arise in quick succession.

At the *posterior margin of the cytostome* the presutural ciliary waves arise. These are confined to the left side of the body between the oral margin and the lateral suture. From the point of origin, waves emerge in a semicircular pattern resembling concentric ripples on a lake. The anterior end of each wave terminates in the apical region; the posterior part, at the lateral suture and the posterior margin of the body.

At the *apical end of the peristome* the waves of the adoral zone seem to be transferred to the postsutural ciliary lines to continue as waves of the postsutural region and the entire right side of the body. Each wave arising at the apex is continuous from the lateral suture on the left side of the body to the aboral border, around on to the right side and over to the oral border, thus covering approximately three-fourths of the circumference of the body. These waves pass to the posterior end of the body, in a pattern which is nearly at right angles to the ciliary rows. The integrity of each wave is not kept up throughout the entire course, each becoming irregular as it nears its terminus.

This appears to be the normal ciliary wave pattern, as it is seen in all movements except the rapid aboral turning (fig. B, 1.). Even in aboral turning this pattern often exists, namely, when the movement is executed slowly (fig. B, 3). When aboral turning is executed rapidly, a reversal of ciliary action occurs in the postsutural region and on the right side of the body. The presutural and membranellar waves are not affected. With this ciliary reversal a rapid change occurs in the ciliary wave pattern of the regions involved. There is a temporary region of confusion in the median aboral section on the left side of the body (fig. A, 2). The normal ciliary wave pattern is interrupted, and new waves arise which create a vortex for the moment and then continue toward the apex. What happens on the right side when ciliary reversal first occurs is not known, as the organisms have never oriented themselves favorably for this observation. However, it is known that the ciliary waves of that side proceed toward the apex as continuations of the postsutural waves by the time they have emerged from the vortex.

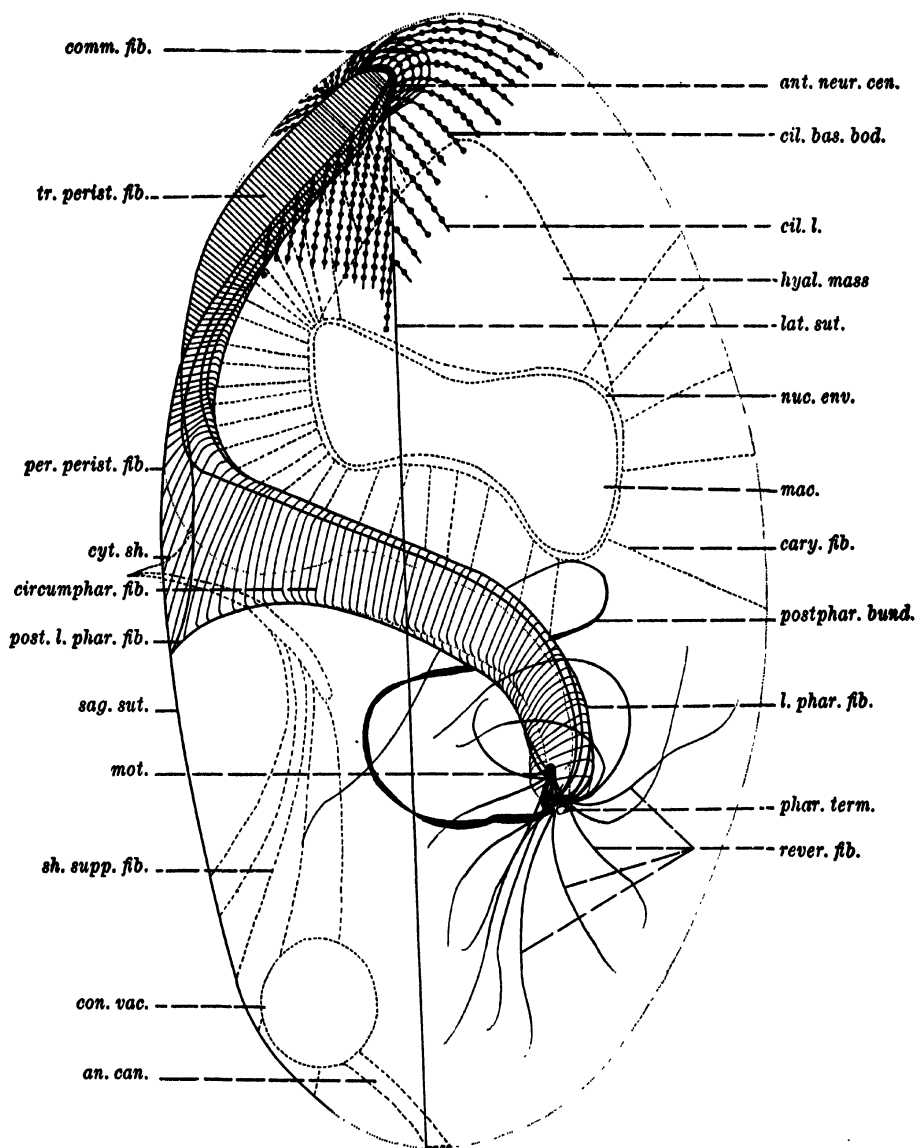


Figure C

Fig. C. A diagrammatic representation of the fibrillar system of *Nyctotherus hylae*. $\times 670$. The left-right bundles have been omitted. Fibrils represented by dotted lines are those thought to be only of a supportive nature. Cilia have been omitted for the sake of clearness, and only parts of the ciliary rows have been drawn for the same reason. Abbreviations: *an. can.*, anal canal; *ant. neur. cen.*, anterior neuromotor center; *cary. fib.*, caryophore fibril; *cil. bas. bod.*, ciliary basal body; *cil. l.*, ciliary line; *circumphar. fib.*, circumpharyngeal fibril; *comm. fib.*, commissural fibril; *con. vac.*, contractile vacuole; *cyt. sh.*, cytostomal shelf; *hyal. mass*, hyaline mass; *l. phar. fib.*, longitudinal pharyngeal fibril; *lat. sut.*, lateral suture; *mac.*, macronucleus; *mot.*, motorium; *nuc. env.*, nuclear envelope; *per. perist. fib.*, peripheral peristomal fibril; *phar. term.*, pharyngeal terminus; *post. l. phar. fib.*, posterior longitudinal pharyngeal fibril; *postphar. bund.*, postpharyngeal bundle; *rever. fib.*, reversal fibrils; *sag. sut.*, sagittal suture; *sh. supp. fib.*, shelf-supporting fibril; *tr. perist. fib.*, transverse peristomal fibril.

The various movements seem to be the result of a differential rate of ciliary action in certain areas. The peristomal membranelles aid in the intake of food, but they are also of appreciable importance as locomotor organelles. When the aboral movement is executed slowly it appears to be the membranelles which mainly bring about the turning, since their rate of beat is increased greatly. In the rapid aboral turning they also beat vigorously, but the reversal of ciliary movement is the important factor in this turning.

The rotatory movements (fig. B, 2, *a-b*) are accomplished by means of the concavity of the body, along with the active beating of the membranelles in the diagonally set peristome. With the clockwise rotation, the peristome is coiled to the left; with the counterclockwise rotation, the peristome has been shifted so that it is coiled slightly to the right.

Postsuturally on the left side, and throughout the right side of the body, the effective stroke of the cilia is in the direction of the wave progression. In the presutural and membranelar waves the effective stroke is opposite to the direction of wave progression; no reversal of beat has ever been noticed in these two regions.

THE NEUROMOTOR SYSTEM

Ten Kate (1927) is the only investigator to date who has published a detailed account of the fibrillar system of *Nyctotherus*. He has worked on two species, namely, *N. cordiformis* and *N. ovalis*. For neither of these did he assign a coördinating function to the fibrillar structures. In *N. hylae* the writer finds a system so well integrated structurally that coördination is a natural corollary. In this organism fibrils are present which do not appear to have a coördinating function, but their presence in no way limits the possibility of integration. Such fibrils may be taken care of under the categories of support, contraction, or, perhaps, a diffuse endoplasmic relation. The neuromotor system of *N. hylae* is not one to be divided into separate units, since it is completely connected and integrated throughout, with its structural relations conforming to fundamental behavior. However, for the sake of convenience the various functional morphological parts of the system will be treated individually.

The components of the neuromotor system are as follows:

- | | |
|---|-------------------------------------|
| I. The <i>motorium</i> | VII. The anterior neuromotor center |
| II. The longitudinal pharyngeal fibrils | |
| III. Reversal fibrils | VIII. Cilia and associated parts |
| IV. Postpharyngeal bundle | a. ciliary lines |
| V. Pharyngeal terminus | b. lateral suture |
| VI. Membranelles and associated parts | c. sagittal suture |
| a. membranelle connectives | d. commissural fibrils |
| b. circumpharyngeal fibrils | IX. Left-right fibrils |
| c. transverse peristomal fibrils | X. Caryophore fibrils |
| d. peripheral peristomal fibril | XI. Shelf-supporting fibrils |

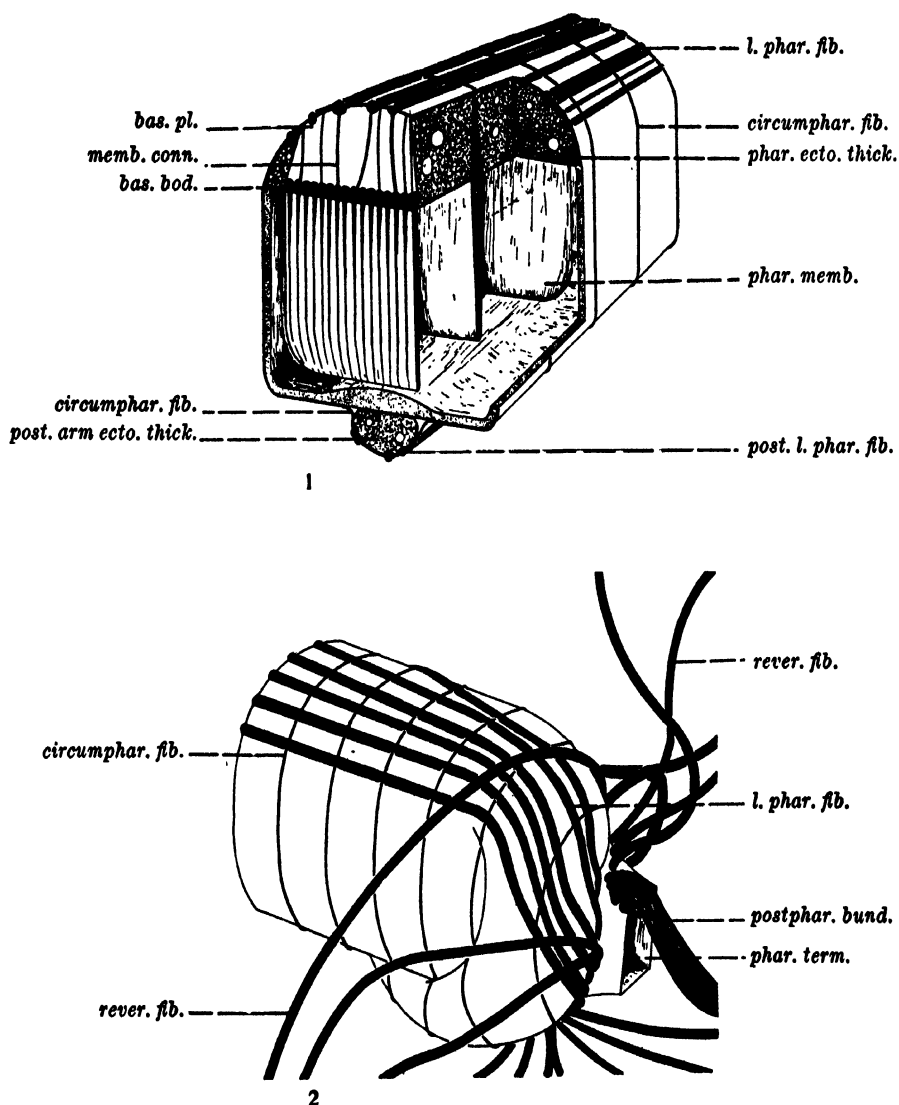


Figure D

Fig. D. Schematic stereograms of parts of the cytopharynx of *Nyctotherus*, ca. $\times 4500$.

1. A section from the central part of the pharynx, showing a part removed and one membranelle cut open to show the cilia. Abbreviations: *bas. bod.*, basal bodies; *bas. pl.*, basal plate; *circumphar. fb.*, circumpharyngeal fibril; *l. phar. fb.*, longitudinal pharyngeal fibril; *memb. conn.*, membranelle connective; *phar. ecto. thick.*, pharyngeal ectoplasmic thickening; *phar. memb.*, pharyngeal membranelle; *post. arm ecto. thick.*, posterior arm of ectoplasmic thickening; *post. l. phar. fb.*, posterior longitudinal pharyngeal fibril.

2. The posterior end of the pharynx, showing the recurving of the ectoplasmic thickening and the pharyngeal terminus opening into the endoplasm. Abbreviations: *circumphar. fb.*, circumpharyngeal fibril; *l. phar. fb.*, longitudinal pharyngeal fibril; *postphar. bund.*, postpharyngeal bundle; *phar. term.*, pharyngeal terminus; *rever. fb.*, reversal fibril.

The *motorium* (fig. C, *mot.*; pl. 14, fig. 2), like the same organelle in other Protozoa, is an ectoplasmic structure, or, at least, is intimately connected with the ectoplasm. The shape of this important organelle in *N. hylae* is roughly that of a slightly irregular carrot, rounded at the broad end, flattened on one side, and tapering to a point at the other end. Its shape and situation have a striking resemblance to the same organelle in other ciliates to be discussed. It is suitably situated for the function it is called upon to perform, namely, that of coördinating the activities of the organism. Such function necessarily calls for a centralized organelle to which all the conductile fibrils may be directly or indirectly traced. Lucas (1934) has described for *Metopus circumlabens* a motorium which in proportionate size, shape, and situation is so similar to that of *N. hylae* that there seems to be little doubt of a close homology. Another neuromotor system closely related to that of *N. hylae* is found in *Conchophthirius magna* (Kidder, 1934). In it there is no description of a neuromotorium, but the "fibrillar bundle" of that organism is undoubtedly the equivalent.

The *longitudinal pharyngeal fibrils* are divided into two groups, each consisting of many fibrils. Both sets arise directly from the motorium and proceed along the pharynx toward the oral border. The larger group is referred to as the *longitudinal pharyngeal fibrils* (figs. C; D, 1-2; E, *l. phar. fib.*; pl. 14, figs. 1-5, 7). They proceed along the anterior border of the pharynx and along the peristome to terminate at the apical end of the latter. The number of fibrils is not uniform; there are usually about ten, but it is not unusual to find as few as eight or as many as twelve or thirteen. This variation may be accounted for by possible branching or by diminishing diameter of fibrils, which are not all the same size. Branching, however, has not been seen. The second group of longitudinal fibrils is called *posterior longitudinal pharyngeal fibrils* (figs. C; D, 1; E, *p. l. phar. fib.*; pl. 14, figs. 1-3, 5, 9), because they are situated along the posterior border of the posterior arm of the ectoplasmic thickening. Here, again, the fibrillar number and size are not constant. Since the arm of the ectoplasmic thickening diminishes as it proceeds toward the oral margin, it is difficult to determine the exact number of fibrils at its terminus (pl. 14, fig. 9), but it is apparent that more than one fibril reaches the cytostomal border to unite with the peripheral peristomal fibril (figs. A, 1-4; C, *per. perist. fib.*; pl. 14, figs. 1-4, 6, 9).

The functions of these two sets of fibrils are somewhat different. The longitudinal pharyngeal fibrils seem to be correlators of the membranelar activity, carrying stimulation from the motorium to the connectives in the pharynx, and ultimately to the anterior neuromotor center, where they terminate. The posterior longitudinals are not connected in any way with membranelles but terminate at the cytostomal border, where the presutural ciliary waves arise, thus giving evidence of function as ciliary wave propagators in that region.

Reversal fibrils (figs. C; D, 2; E, *rever. fib.*; pl. 14, figs. 1-3) is the designation given to a group of fibrils radiating from the distal end of the cytopharynx, through the endoplasm, to unite with the ciliary lines at several

points, exclusive of the presutural ciliary lines. Their name is significant of the function they are thought to perform, namely, that of ciliary reversal. These fibrils arise both from the motorium and the pharyngeal terminus. Entz, Jr. (1913), found these fibrils in *N. piscicola* but did not ascribe this function to them. Ten Kate (1927) found the same fibrils in *N. ovalis*. In *N. cordiformis* he found them to be sparsely represented and failed to find their connection with the outer margins. He, also, failed to assign to them any power of conduction.

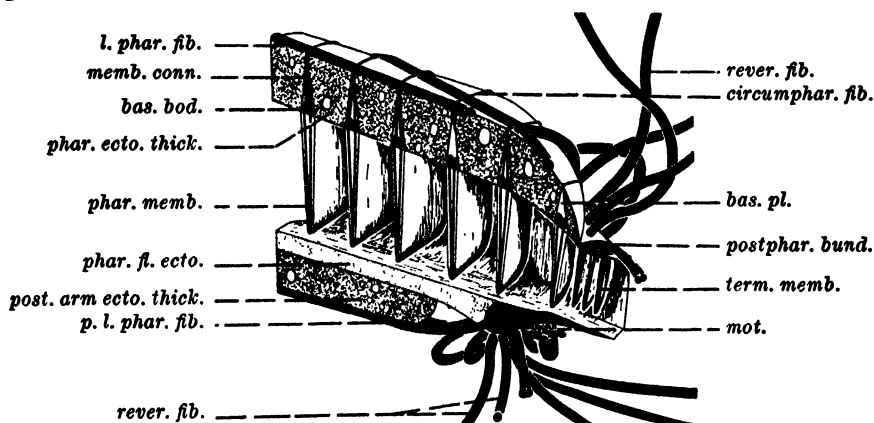


Figure E

Fig. E. Schematic stereogram of the posterior end of the cytopharynx, showing the internal structure of Fig. D, 2. Abbreviations: *bas. bod.*, basal bodies; *bas. pl.*, basal plate; *circumphar. fib.*, circumpharyngeal fibril; *l. phar. fib.*, longitudinal pharyngeal fibril; *memb. conn.*, membranelle connective; *mot.*, motorium; *phar. ecto. thick.*, pharyngeal ectoplasmic thickening; *phar. memb.*, pharyngeal membranelles; *phar. fl. ecto.*, pharyngeal floor ectoplasm; *p. l. phar. fib.*, posterior longitudinal pharyngeal fibril; *post. arm ecto. thick.*, posterior arm of ectoplasmic thickening; *postphar. bund.*, postpharyngeal bundle; *rever. fib.*, reversal fibrils; *term. memb.*, terminal membranelles arising from the pharyngeal terminus. $\times 4500$, approximately.

The *pharyngeal terminus* (figs. C; D, 2; E, *phar. term.*; pl. 14, fig. 2) is the short, constricted distal part of the cytopharynx. This structure has an intense affinity for the haematoxylin stain, taking as deep a stain as does the motorium, to which it bears an intimate and important relation. The three terminal membranelles (fig. E, *term. memb.*; pl. 14, fig. 2) arise directly from this terminus. Distinct basal bodies for these membranelles may be present, but the terminus stains so darkly that they cannot be distinguished. The terminus probably corresponds to the "oral ring" of *Boveria teredinidi* (Pickard, 1927); the "circumoesophageal fibril" of *Balantidium coli* (McDonald, 1922); the "circumoesophageal ring" of *Diplodinium ecaudatum* (Sharp, 1914), *Tintinnopsis nucula* (Campbell, 1926), and *Favella serrata* (Campbell, 1927); the "pharyngeal ring fibril" of *Conchophthirius magna* (Kidder, 1934); and, perhaps, to the "fibrillar ring" of *Haptophrya michiganensis* (Bush, 1934). In the above-named organisms it is not uncommon to find that the ring structure, as well as the motorium, is also a place of origin of fibrils. In *N. hylae* the postpharyngeal bundle arises entirely from the terminus, whereas reversal fibrils arise from both the terminus and the motorium.

The *postpharyngeal bundle* (figs. C; D, 2; E, *postphar. bund.*; pl. 14, fig. 3) is a group of fibrils arising from the pharyngeal terminus and proceeding in the endoplasm in an irregular course, diminishing in size as it continues. Its termination at its distal end is without visible attachment. The fibrils of this bundle are closely packed, so much so that the fibrillar structure is not always apparent. The first report of this structure for *Nyctotherus* was made by Higgins (1929), who misinterpreted it as a tube, named by her the "continuation tube," probably because a cross section of the bundle occasionally shows up as a tube. The species on which Higgins worked was *cordiformis*. In her opinion, the continuation tube is the extension of what she called the "subpharyngeal canal." So far as the writer has been able to find, no such structure exists in *hylae* or *cordiformis*. The subpharyngeal canal is figured in the same situation as the posterior arm of the ectoplasmic thickening (figs. D, 1; E, *post. arm ecto. thick.*; pl. 14, figs. 1-3, 5, 9). One can easily understand how this mistake might be made, as continuity between this posterior arm and the postpharyngeal bundle might be apparent to one who had not studied sections. Higgins does not figure any sectioned material, nor does she mention having approached the problem from this point of view. Had sections been studied it seems improbable that a subpharyngeal tube would have been found continuing beyond the distal end of the cytopharynx as a continuation tube. Further, Maier (1903) and Ten Kate (1927) already had figured this rodlike arm of the ectoplasmic thickening. The ampule-like structure which Higgins has figured at the central part of the tube is the region in which the ectoplasmic thickening turns over the distal end of the cytopharynx to proceed forward on the posterior side of it.

Kirby (1932) found the postpharyngeal structure in *N. silvestrianus*. He, too, was unable to find a canal structure on the underside of the pharynx. The postpharyngeal organelle in *N. silvestrianus* is a band-shaped filament, probably fibrillar as in *N. hylae*, extending from the end of the cytopharynx into the endoplasm, where its position varies in different specimens, since it is not attached at its distal end. This observation is in full accord with that made by the writer on specimens of *N. hylae*.

Lynch (1930) described a similar postpharyngeal structure in *Lechriopyla mystax*, called the "pellicular fiber," in which there was seen a definite fibrillar structure having the form of a flat band as it leaves the cytopharynx, and made up of three or four heavy fibrils united by a delicate membrane composed, in turn, of delicate longitudinal fibrils. This delicate group of fibrils has not been seen in *N. hylae*. In all ciliates in which it has been found, this postpharyngeal structure has been described as free at its distal end and as diminishing in size from its origin to its free end. The one exception is the description by Higgins (1929), who stated it to be a tubelike structure often terminating in the contractile vacuole. Early investigators of *Nyctotherus* did not discover the presence of a postpharyngeal bundle. Maier (1903), working with *N. cordiformis*, Entz, Jr. (1913), with *N. piscicola*, and Ten Kate (1927), with *N. cordiformis* and *N. ovalis*, did not mention this structure. In

each paper, however, there was some indication of its presence. Each figured a short continuation of the pharynx longer than the pharyngeal terminus. Entz, Jr. (1913, Pl. II, fig. 2), shows a heavy, dark continuation of the pharynx which comes to an abrupt end after a short distance. In another part of the endoplasm there appears to be a part of a tortuous fibril which closely resembles a part of the postpharyngeal bundle as found in *N. silvestrianus* and *N. hylae*. Entz makes no mention of this tortuous structure, but since his photographs are of sectioned material, it is quite likely that this tortuous fibril is identical with a part of the postpharyngeal bundle. Kidder (1934) figures a "gullet fibril" which follows the floor of the gullet of *Conchophthirius magna* and is comparable, in situation at least, to the postpharyngeal bundle of *N. hylae*. The "pharyngeal strand" of *Metopus circumlabens* (Lucas, 1934) resembles the bundle of *N. hylae*, but is described as arising from the motorium and not from a ring structure comparable to the pharyngeal terminus. One might expect this variation, however, between two different genera.

To assign a function to the postpharyngeal bundle is a difficult task, since it is not visibly connected at its distal end with any motor organelles of the body. However, its intimate association with the endoplasm indicates some relation to the endoplasmic activities associated with the metabolic processes of the organism.

Membranelles are plates formed by the fusion of two rows of cilia. Fixation usually causes the plate to break up so that its individual components are seen. The double row of basal bodies is easily distinguished (figs. D, 1; E, *bas. bod.*; pl. 14, figs. 2-3, 7-8). From the base of each membranelle there is a wedge-shaped basal plate which is imbedded in the ectoplasmic thickening and which tapers to a fine edge at the endoplasmic boundary of the thickening. Within the plate there are the fine fibrils which are called the *membranelle connectives* (figs. D, 1; E, *memb. conn.*; pl. 14, fig. 5). These arise from the basal bodies and connect with the circumpharyngeal fibrils, and are in communication with the motorium through them.

The *circumpharyngeal fibrils* (figs. C; D, 1-2; E, *circumphar. fib.*; pl. 14, figs. 1-2, 7-9) are a series of fine fibrils which encircle the pharynx throughout its length, one at the level of each membranelle. They penetrate both sets of longitudinal fibrils. In the peristome, the circumpharyngeal fibrils are replaced by the *transverse peristomal fibrils* (fig. C, *tr. perist. fib.*; pl. 14, figs. 2-3, 6). These are more abundant in relation to the membranelles than are the circumpharyngeal fibrils. For each membranelle there are at least two transverse fibrils.

This great group of circular and longitudinal fibrils creates an intricate basket, with which the membranelles are intimately associated. The membranelles are thus coordinated into a system of effective ingestatory as well as locomotor organelles. The basket thus formed is quite similar to the "pharyngo-esophageal net" of *Paramecium multimicronucleata* (Lund, 1933). Kidder (1934) describes for *Conchophthirius magna* an intricate pharyngeal and peristomal basket which is even more like that of *N. hylae* than is that of

Paramecium. Kidder does not figure a complete *peripheral peristomal fibril* such as is present in *N. hylae*. The pharynx of *Conchophthirius* possesses only a single row of long cilia and the peristome lacks membranelles or cilia. The entire structure is capable of contraction, a condition not present in *Nyctotherus*.

The *anterior neuromotor center* (figs. A, 3; C, *ant. neur. cen.*; pl. 14, fig. 4) is at the apical end of the peristome. It has a fibrillar appearance, apparently being composed of a number of the longitudinal fibrils which have continued

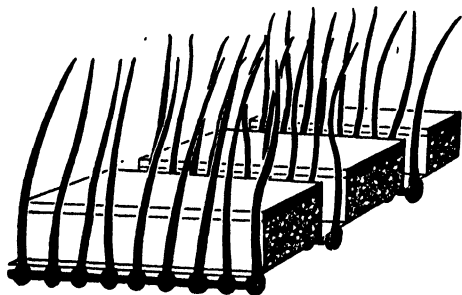


Figure F

Fig. F. Schematic stereogram of a part of the pellicle of *Nyctotherus hylae*. $\times 3900$. This figure shows the relationship of the basal bodies to the ectoplasmic layers. The basal bodies are connected longitudinally by the ciliary lines. The cilia are seen to emerge from the regular furrows in the pellicle.

from the motorium proper. Many ciliary lines arise directly from this center. The anterior neuromotor center is only a supplement of the motorium, as the two are directly connected.

Each *ciliary* line of the body surface connects a series of basal bodies in an apical-antapical row (figs. A, 3-4; C, *cil. l.* and *cil. bas. bod.*; pl. 14, figs. 1-4, 6-9). The cilia are of varying lengths in different regions of the body, the average length being 12μ . Each cilium arises from a basal body found in the endoplasm just below the ectoplasm, which is

in two layers, the outer one being very dense. The relationship of the basal body to the double-layered ectoplasm is seen in almost any cross section of the body (fig. F.; pl. 14, figs. 6-9). Coördination of ciliary movement is brought about by the ciliary lines, for the most part, as the waves progress along them in all except the presutural regions. The ciliary basal bodies in themselves are possibly storehouses of energy. This supposition is based on the fact that during the *axial gradient* experiment, although coördination was lost after the toxic agent became effective, probably because of destruction of the neuromotor centers, ciliary movement nevertheless continued for some time.

The *lateral* and *sagittal* sutures divide the ciliation of the body into definite regions and probably act as conductors between ciliary lines, as well as boundary lines.

Commissural fibrils have been found only in the apical postsutural region (figs. A, 3; C, *comm. fib.*; pl. 14, fig. 4). This series of four fibrils serves to coördinate the ciliary movement of the postsutural region into definite unitary waves. Each wave begins to lose its integrity as soon as it passes the last commissure, so that by the time it reaches the posterior end of the body it is quite irregular. The commissural fibrils are connected with the motorium through the ciliary lines and the neuromotor center at the apex of the body which is connected with the motorium by the longitudinal fibrils.

Since commissural fibrils have not been found in the presutural region, it

is less easy to explain the integrity of the presutural waves. Propagation of these waves occurs at the posterior margin of the cytostome where the posterior longitudinal pharyngeal fibrils terminate. No concentration of neuromotor elements similar to the anterior neuromotor center is found here. Stimulation for the propagation might come from the motorium through these posterior pharyngeal fibrils and travel either by commissures, which present technique fails to show, or by the peripheral peristomal fibril and sagittal suture. The second explanation is inadequate, but, on the assumption that fibrillar connections are the mechanism of nervous conduction, it is the only one available if commissures are absent.

The *left-right fibrils* (pl. 14, figs. 1-3, 6-7, 9) so prominent in *Nyctotherus* are best interpreted as morphonemes, serving to retain the left-right compression of the body, for which function the unaided pellicle would probably be insufficient. There is possibly a certain degree of contractility in these fibrils to aid in the formation of the concavity of the body during rotatory movements. However, their action on that basis would be hard to explain, as contraction would cause double concavity rather than concavo-convexity, as is actually what occurs.

The caryophore fibrils (fig. C, *cary. fib.*; pl. 14, figs. 1-2, 6) support the nuclei. They are attached to the ciliary lines, the transverse peristomal fibrils, and the circumpharyngeal fibrils, at their outer ends, and to the nuclear envelope (fig. C, *nuc. env.*) at their inner ends. The nuclear envelope is composed of a fibrillar network into which these fibrils insert.

The *shelf-supporting fibrils* (fig. C, *sh. supp. fib.*; pl. 14, fig. 2) are also, supposedly, a group of morphonemes. They are attached to the base of the oral bristle at the one end and to the ciliary lines at the other. The base of the oral bristle is an expanded left-right ectoplasmic anchorage supported in part by a group of left-right fibrils (pl. 14, figs. 1-3).

DISCUSSION

Nyctotherus hylae possesses numerous fibrils bearing to it the same relationships as do the fibrils of many other ciliates. The movements of this heterotrich are well coördinated, producing characteristic vital patterns under various conditions of stimulation. It is pertinent to ask through what mechanism this coördination and integration can be accomplished. To the writer, there is no choice but to accept the neuromotor hypothesis and thus relegate the coördinative functions to the intricate fibrillar system.

The "skating" movements of the organism are those most generally encountered (fig. B, 1, 3-4). The first of these, the one in which the organism moves forward at an angle of 40 degrees to the left of the main axis (fig. B, 4), is the one most frequently observed. It involves the normal patterning of the ciliary waves, coördinated by the intricate neuromotor mechanism. The membranelles are active at this time but do not seem to be prominent organelles of locomotion for this particular movement. The ciliary wave pattern existing at this time would naturally cause the organism to move at the angle at which

the waves progress. The presutural waves, with the membranellar waves, function to retain the axis of the body at the angle of progression.

The second frequent movement is the aboral circling in a large orbit (fig. B, 3). At this time the normal ciliary wave pattern is still retained, but a greater membranellar activity is noticed. The enhanced activity of these powerful membranelles tends to turn the organism aborally, while the body cilia propel the organism sufficiently to cause the circumscription of a large circle. The coördination of activity between the motorium and the anterior neuromotor center probably increases membranellar activity while the normal activity of the body cilia is retained.

The third of the skating movements involves the reversal of ciliary action previously mentioned (fig. B, 1). In this movement the organism makes no forward progression but spins aborally with great rapidity, circumscribing a circle with a radius equivalent to the length of the body. The activity of the presutural cilia remains normal, as it does in every other movement executed. The membranelles are sped up noticeably, but the aboral spinning is further aided by the reversal of ciliary action in the postsutural region and on the right side of the body. As membranelles impel the oral border of the body forward, the reversed cilia cause the aboral parts to move posteriorly, thus causing the spinning. This motion occurs when the organism meets some obstacle or approaches the limits of its confines, where conditions of oxygen tension and other factors may be adverse. Stimuli are probably conveyed to the motorium by certain of the longitudinal fibrils of the pharynx, so that motor stimuli are set up, some of which increase activity of the membranelles and others of which travel out to the ciliary lines by way of the reversal fibrils, to create the reversal of ciliary motion.

The rotatory movements of the organism are unique (fig. B, *a-b*). Of the two rotations, the clockwise one is the more common. The normal ciliary wave pattern exists for both, but the cupping of the body and the position of the peristome are the two differential factors of importance. The cupping is caused by some sort of contractile element in the body, but it is difficult to assign this function to any of the fibrils. The most plausible assumption is that the ciliary lines are contractile as well as conductile. The contracted body forces the peristome to assume a position which is more angular than normal, its apex lying far up on the convex side of the body. This creates a groove which sets the membranelles at an angle so that their action serves to pull the body in a clockwise direction. The reverse condition obtains when the counter-clockwise rotation is performed. Cupping creates a concavity of the left side with a convexity on the right. The peristome becomes appreciably shifted so that it is slightly angular on the right side of the oral border. It assumes this position not so much by the contractile ciliary lines attached to it as by the pushing caused by the concavity on the left side. However, it is not pushed so far to the right proportionately as it is pushed to the left at the time of its clockwise rotation.

The feeding necessities of this organism are adequately cared for by the

membranellar action, which serves to bring a constant stream of bacterial food to the cytostome. Here, under the guidance of the well-supported cytostomal shelf, it is turned into the cytopharynx. The pharyngeal membranelles carry as much of it as possible to the distal end of the pharynx, and the terminal membranelles give it a last push into the endoplasm. In the endoplasm its course is unknown, but endoplasmic activity is probably coördinated to care for it, perhaps by the postpharyngeal bundle.

SUMMARY

1. *Nyctotherus hylae*, heretofore known as *Nyctotherus cordiformis* variety *hylae* Stein (1867), is given full specific status based on the original description by Stein and on comparisons of the fibrillar systems of the two species. The relative sizes and shapes of the two species are different, *N. hylae* being larger and more egg-shaped than kidney-shaped. *N. hylae* possesses a hyaline mass anterior to the macronucleus, a condition not present in *N. cordiformis*. The fibrillar system of *N. hylae* is more advanced than that of *N. cordiformis*.

2. The motor activities of *N. hylae* have been studied by anatomical and cinematographic methods in order to interpret more adequately the interrelations of the fibrillar system and movements. It was found that the organism is capable of a certain amount of contraction, a phenomenon not previously reported for any species of *Nyctotherus*. Contraction causes a cupping of the body, either on the right or left side, depending on whether the organism is rotating clockwise or counterclockwise. The ciliary lines are thought to be responsible for the contraction. Reversal of ciliary action is present but is restricted to certain parts of the body. Presutural cilia and membranelles never reverse their activity.

3. The organism was found to possess a positive thigmotropism and a positive geotropism, or perhaps equilibrium response.

4. The oral bristle described by Stein in 1867 and verified by later investigators was found to be only a part of a larger structure, the cytostomal shelf. The shelf serves to direct food into the pharynx, acting as a passive agent only. This function is the same as the one Stein had attributed to the oral bristle.

5. It is shown that an axial gradient exists, which establishes the morphological anterior end as the physiological anterior end.

6. The neuromotor system has been found to be structurally integrated throughout the body. Whatever its function is, conduction and coördination seem to be its chief rôle, because the movements of the body are such as would result from a well coördinated system only. A distinct motorium is present at the distal end of the cytopharynx in the ectoplasmic layer. A secondary center, termed the anterior neuromotor center, occurs at the apical end of the peristome, and seems to coördinate with the motorium proper. Membranellar waves arise from the region of the motorium, whereas ciliary waves arise at points connected with important extensions of the motorium. Certain fibrils arising from the motorium and terminating in the ciliary lines are thought to be conductile agents responsible for reversal of ciliary action. A postpharyn-

geal bundle is described, which is morphologically equivalent to similar structures in other ciliates. It is not visibly connected with motor organelles, and therefore is assigned a diffuse endoplasmic relation. Certain fibrils which connect the right with the left side of the body are considered to be morphonemes, serving to retain the left-right flatness of the body. What there is of contractility is assigned to the ciliary lines.

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EXPLANATION OF PLATE

All figures are of *Nyctotherus hylae*, drawn with the aid of a camera lucida from preparations fixed in Schaudinn's fluid and stained with Haidenhain's haematoxylin. Magnification of 520 diameters unless otherwise stated.

PLATE 14

Figs. 1-4. A series of consecutive sagittal sections, the first one being nearest the right side; fig. 4 is only the anterior end of the organism. Fig. 5. A cross section of the cytopharynx about midway between the cytostome and the distal end of the pharynx, $\times 2080$. Figs. 6-7. Consecutive sections, the first one being the more anterior. Fig. 8. The same section as fig. 7 but drawn at a lower focus. Fig. 9. A section posterior to the cytostome viewed from the opposite pole.

Fig. 1. Reversal fibrils are clearly shown at the posterior end of the body as they join the ciliary lines. The oral bristle arises from its supporting base and protrudes from the cytostome. Only a small part of the macronucleus occurs in this section. The hyaline mass is shown as a slight dispersed area anterior to the macronucleus. The micronucleus is the black, vesicular body at the posterior limits of the macronucleus. A few caryophore fibrils are present. The dark mass at the posterior border of the oral bristle is a cross section of the left-right fibrillar bundle in the region. A few membranelles are shown.

Fig. 2. Most of the structures present in the preceding section are found here also. The fibrillar nature of the nuclear envelope is evident. There are many circumpharyngeal fibrils. In one part the pharyngeal structure is well shown. The motorium, pharyngeal terminus, terminal membranelles, and reversal fibrils occur at the distal end of the pharynx. There are a few shelf-supporting fibrils, a few transverse peristomal fibrils, and a part of the peripheral peristomal fibril. The contractile vacuole and the anal canal are prominent.

Fig. 3. The fibrillar nature of the postpharyngeal bundle is evident; longitudinal pharyngeal fibrils showing clearly. The dense hyaline mass and the chromophilic basal plates are shown. The anal canal is at an angle different from that of the previous section.

Fig. 4. This section is to show chiefly the relationships of the anterior neuromotor center, the longitudinal pharyngeal fibrils, the commissural fibrils, and the ciliary lines.

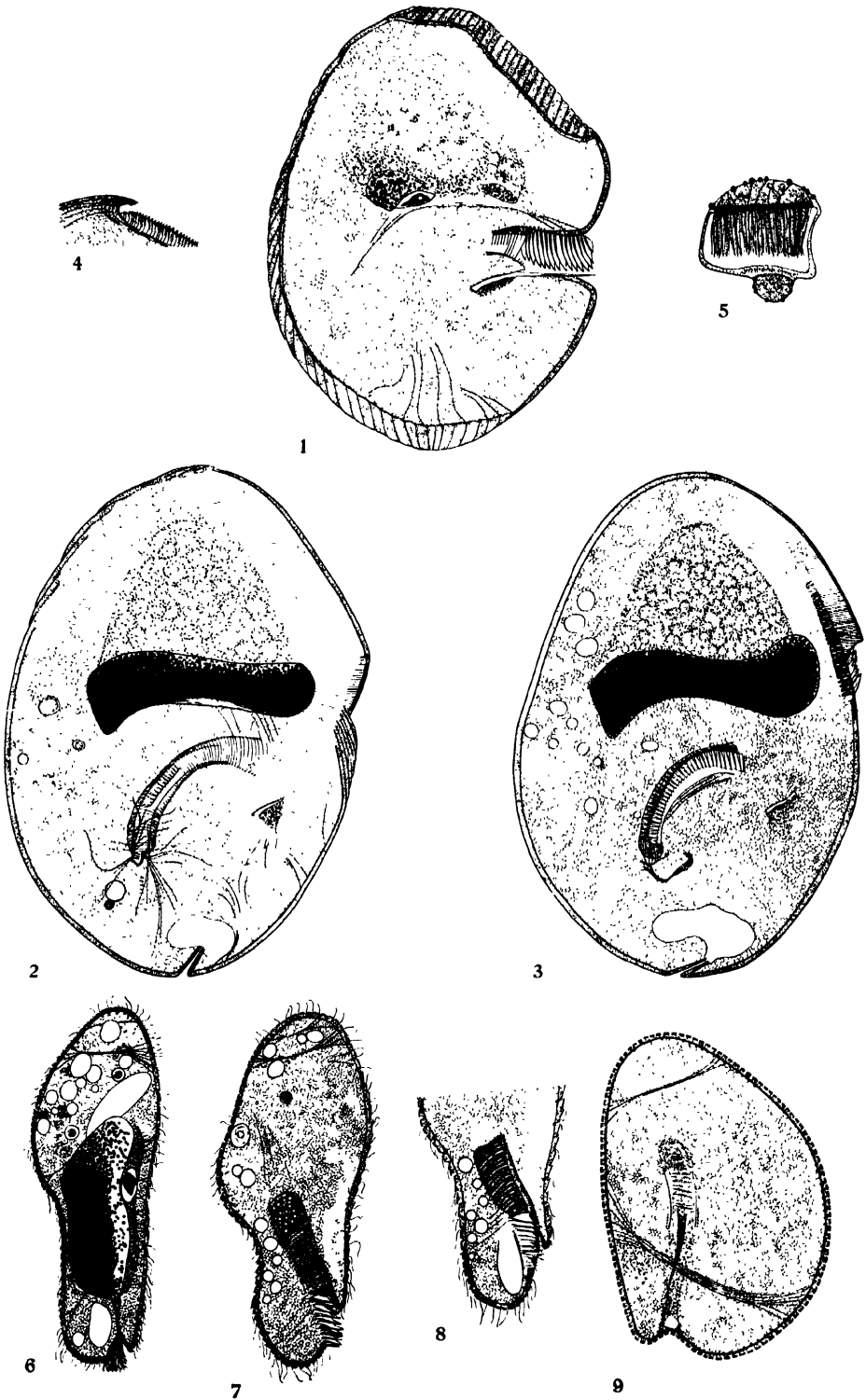
Fig. 5. Showing the relationships of this part of the cytopharynx as schematized in text figure D, 1. The basal bodies appear as a continuous plate rather than separate entities. They are joined by several membranelle connectives to the longitudinal pharyngeal fibrils.

Fig. 6. Left-right bundles are seen in the aboral region. Caryophore fibrils are numerous. A part of the hyaline mass is present on one side of the macronucleus, in which the micronucleus is imbedded. Basal bodies, cilia, and parts of the peripheral and transverse peristomal fibrils may be seen.

Fig. 7. Circumpharyngeal fibrils, a few longitudinal pharyngeals, a few of the membranelle basal bodies, and several membranelles.

Fig. 8. Showing, chiefly, the basal bodies of the membranelles.

Fig. 9. Two main groups of left-right fibrils. The posterior arm of the ectoplasmic thickening tapers to a point to meet the peripheral peristomal fibril. A few cross sections of circumpharyngeal fibrils may be seen. Cilia have not been drawn.



GENE AND CHARACTER
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THIS PAPER supplements a former publication on the same subject, part III of this series (Goldschmidt, 1935: Biol. Centrbl.; *not* Univ. Calif. Publ. Zoöl.). In that paper the development of the wings of a number of mutants and alleles of *Drosophila melanogaster* was described, which had in common a more or less extensive scalloping effect upon the wing margin (Beadex, Beadex^J, vestigial, vestigial-nicked, cut). It was shown that in all these types the entire wing developed normally up to a certain stage and that after this stage more or less extensive parts of the margin of the wing became abnormal and were resorbed. The greater the damage to the wing, the earlier the onset of the abnormality of the tissue, so that in the type vg, where the greater part of the wing, except its base, is resorbed, the destructive process must be supposed to start in early larval stages. The best material for the study of this seriation in time is found in the series of vg alleles which show the scalloping of the wings from a small nick through all intermediate stages, up to an almost complete disappearance of the wing. In the earlier paper only vg and vgⁿ¹ were studied, and the intermediate stages were derived from the related Bx series. This gap will be filled first in this paper.

THE vg-ALLELE COMPOUNDS

We had for this purpose a very useful line, namely, a compound between vestigial and notched vg/vg^{no}. This compound, derived from Mohr's stock and bred for a long time in mass culture, showed a much larger variation than usual. As a matter of fact, most of the higher classes of scalloping were represented, from notched (classes IV-V) up to almost vestigial (class X), with a majority of the types ragged, and strap (classes VII, VIII). Within this compound, therefore, the development of all intermediate types could be studied. In the former paper, part III, it was emphasized that soon after pupation the wing grows to its pupal size and that at this moment a chitinous sheath is secreted, which represents an exact mold of the wing at this stage. Thus, further developmental stages can always be referred to this stage, the contour of which is always known from the condition of the sheath.

It is not necessary to go into the details of the processes of degeneration and resorption, which have been described in part III. We illustrate simply the development of the intermediate stages between little scalloping (classes I-III) and vestigial (class X). Plate 15 represents three stages of the pupal wing development, to illustrate the maximum destruction of wing area which might take place when, after pupation, the wing form, as indicated by the

contour of the sheath, is still completely normal. The wing here under consideration belongs to class V of scalloping. Plate 16 represents three similar stages, but in these the resorption had already begun before pupation, as the wing contour is somewhat irregular. But the damage is as yet rather small; the final wing will belong to about class VI of scalloping. Plate 17 shows a representative set of photographs of three stages of development of a wing belonging to classes VII–VIII. Here the destruction of the wing margin must have set in a rather long time before pupation. The wing at the moment of the secretion of the sheath has already lost rather large parts toward its tip, with the consequence that the wing after its first pupal expansion, preceding the time of sheath formation, shows a bizarre form, varying more or less in detail in different individuals. The form of the sheath which is secreted at this stage again indicates in the photographs of the later stages the condition after pupation and the course of the further resorption of the wing surface. The step from this latter type to vg, figured in the former paper, is not very great and therefore no further intermediate stages need be described.

OTHER MUTANTS

Before scalloping is discussed, a few notes may be added with respect to other wing mutants. In a paper on cell size, Dobzhansky (1929) states that miniature wings have the same number of cells as the wings of wild type, but that they are smaller. This fact can easily be demonstrated by counting the bristles of the anterior margin of the wing and noticing their size and distance apart. The embryological explanation is a very simple one. As reported in an earlier paper, the differentiation of the wing surface is completed before the growth and folding of the wing within its sheath begin. This growth then is, at least as far as the epithelium is concerned, a growth by intussusception without cell division. The miniature wing, which more or less omits this growth period, therefore has smaller cells. The gene *m* prevents this postembryonic increase in cell size.

Besides the miniature wing, we studied the dumpy wing, a wing with a pointed tip from a new mutation, and the expanded wing. Plate 18 represents stages of development of the dumpy wing. At the time of pupation the wing is perfectly normal. Later, we again find degenerating tissue appearing. But this time it is not the epithelium which shows abnormal behavior. As far as can be judged from total mounts, the epithelium remains perfectly normal throughout. The whole subepithelial tissue toward the wing margin appears abnormal and stains differently. After some time the wing margin begins to retract, indicating that the abnormal tissue below the epithelium is being resorbed. This resorption and retraction continue until the dumpy shape is completed. In the photographs, the contour of the sheath again serves as a marker for the condition after pupation. The detailed histological study has still to be made.

It seems that the wing with pointed tip originates in the same way (pl. 19). At the time of pupation the shape of the wing is normal, and only later is the pointing accomplished. In this mutant, however, it is difficult to state whether

a process of resorption is also involved, since the area which is changed is so small that only a detailed histological examination could settle this point.

Very different from the development of all mutants thus far studied is that of the large and broad expanded wing. As plate 20 shows, this wing has already attained its large size and shape at the time of pupation, and otherwise shows no abnormality. Here, then, the mutation results in a larger, but perfectly normal, growth of the imaginal disk. This is an important fact which probably explains the specific behavior which my former student Csik (1934) found for the action of the expanded wing in different combinations of genes affecting wing size. But this question will not be discussed here.

THE WORK OF AUERBACH

After the completion of this study there appeared a paper by Auerbach (1936) which supplements this work in some important respects. Auerbach made a special study of the prepupal stages of the normal and vestigial wings from the first appearance of the imaginal disks. The important points for our problem are: In the early third instar the material of the imaginal disks is divided into thoracic and wing material. At this stage it seems that the circular mass of epithelium, which later will be pushed out into the wing pouch, already is smaller in *vg*, though the whole bud of which it is a part is not. When the disk is evaginated to a pouch and this pouch grows out, it is much smaller than in a normal wing and a part is constricted off by a fold. At this point the description in my former paper applies.

Similarly, the development of *Beadex*^J (pointedoid of Jollos) was studied, the wing of which is very similar to classes VII–VIII (Goldschmidt, 1935). Here the early disk, before the formation of the wing pouch, is perfectly normal. When the pouch is formed and differentiates, its size appears smaller in the direction which will later become the breadth of the wing.

Here, then, is found proof for the condition of the wing anlage, which we had extrapolated from the later stages in the series. Of course, we have no knowledge of the process which decreases the size of the rudiment either later (*Beadex*^J) or earlier (*vg*) in larval life. One would expect it to be of the same type as after pupation, namely, destruction of parts already formed, an expectation which is derived from the fact that determination of the entire wing must have occurred still earlier (Goldschmidt, 1935). But it is also possible that in *vg* the first anlage is smaller. If this is so, the *vg* differs from the rest of the series, which begins with a normal anlage; but we could not understand how the parts of this wing are determined embryonically. However, it will be very difficult to prove histologically the resorption process in the very early imaginal disk. It ought to occur across a diameter of the disk which later will be pushed out as the edge of the flat pouch, the prospective wing margin.

DISCUSSION

The strange and rather unexpected facts concerning the development of the wing in the series of *vg* alleles suggest an interpretation which might be of general importance for the problem of the action of the gene. The following

points have to be brought into line: (1) Scalloping of all grades from a nick to the stump in the vestigial series is the result of resorption of previously formed wing tissue of an originally complete wing. (2) The determination of the wing structures (in the sense of experimental embryology) with all their details in structure and position must have been completed before degeneration and resorption set in; the wings of this series are in fact parts of whole wings, with all remaining structures perfect and in proper position. (3) The larger the amount of destruction of wing area, the earlier in development have involution and resorption set in. (4) The abnormal parts to be resorbed show a definite pattern, which, allowing for a certain amount of individual variation and also a certain control by modifying factors, follow the typical series of the phenotypes of the mutants nicked, notched, snipped, ragged, antlered, strap, vestigial, and no-wing. (5) The two wings vary more or less independently. It is possible for one wing to show grade V and for the other to be normal, but usually both show similar but not symmetrical reductions. (6) The smallest change usually affects one wing only, beginning with the loss of one or two hairs at the wing tip. (7) The pattern in question must be laid down somehow in the mechanism of wing development; the same pattern of reduction is followed, whether the scalloping is caused by the *vg* alleles, or by their compounds, or by the action of dominigenes, or as an effect of temperature shocks (phenocopy), or by breeding *vg* flies under definite temperature conditions. (8) The pattern, however, is specific for definite genes; for example, the genes beaded and beadex and their alleles produce a similar series of wing reduction by resorption, but with a different pattern. (9) The action of the genes and alleles in producing the series of effects, typical in quantity and pattern, is an orderly one of a simple linear nature, as will be proved in a subsequent paper.

If we try to correlate these facts we are reminded of another development which has more than superficial similarity with this one, namely, that of the pattern on the wings of intersexual males of *Lymantria dispar*. Here are found patches of female white scales on the brown male wing (see illustrations, Goldschmidt, 1923). The amount of white is exactly proportional to the degree of intersexuality, and therefore a continuous series of forms exist beginning with a tiny splash of white scales on one of the four brown wings, and ending with all wings white, with perhaps a small streak of brown. This series of gradations of the white encroaching upon brown is asymmetrical on the right and left sides and subject to noticeable individual variation; but a general pattern is easily observed. It is best described in a simile. Looking over a series of these wings the impression is that starting from the base of the wing a sluggish stream of brown color had slowly spread over the wing surface following the direction of the wing veins, and that this stream had been stopped at different stages before it covered the entire wing. It is known, further, that the relative amount of brown and white, that is, the different degrees of the quantitatively variable pattern, is typical for different genetic constitutions in which a series of multiple alleles and modifying factors is involved.

The study of the development of this pattern has revealed (see Goldschmidt,

1923) that a long time before any pigment appears the pattern already exists in the form of a difference in rate of growth of the scales; the differentiation of the future white female scales has progressed beyond that of the future brown ones. The pattern, then, is primarily a matter of growth differences. In this developmental process the time element enters in the following way: Male intersexes are individuals which begin development as males but change sex at a certain turning point, to finish development as females. The earlier the turning point, the higher the degree of intersexuality. The wing pattern, however, changes parallel to the grade of intersexuality in favor of the white parts. This means, therefore, that with an earlier turning point there remains less brown, the original genetic color. That the brown male parts remain at all, however, proves that they were already finally determined when the turning point arrived. From this it follows that the determination of wing-scale form and color in this developmental process proceeds from the wing base in the form of a determination stream spreading sluggishly over the surface of the wing in a pattern, which is preserved in the form of the brown parts in the series of intersexual males from the highest down to the lowest grade of intersexuality. As we expressed it before, this series of intersexual wing patterns may be compared to a series of moving pictures of the progress of the determination stream. As is well known, the idea of a determination stream spreading from a definite center was first used by Spemann to explain the time relations of determination relative to the distance of the cells from the organizing center. One might of course also use the expression "gradient of determining stuff."

It is now possible to apply the general line of reasoning, which in *Lymantria* was derived step by step from the facts, to the vestigial series. In this the pattern of the series of stages is represented by the series of increasing destructions of wing area. If we use the same plastic description as before, we may call the series from vg through strap, antlered, and so forth, to nicked and wild type also a moving picture of the progress of something. This something may be considered in two ways: either as the increase of the degenerating area from nicked to vg, that is, from wing tip to base, or as the increase of the normally developing area, that is, from base (vg) to tip (nicked). In the problem of the intersexual wing was involved a time element, that is, the position of the turning point, which determined the amount of brown area to be formed. In the present investigation the time element, which determines the size of the normal (or the degenerated) part of the wing, is the time of the beginning of the resorption process, earlier or later in development. Therefore, just as the relation of time of action to pattern in the moth wing led to the assumption of some substance spreading slowly over the wing area as the determination stream, or in the form of a gradient, we may link the corresponding facts in *Drosophila* with the assumption that something spreading from base to tip of the wing in a definite path brings about the series of events; the path is made visible in the "moving picture" of the phenotypic series. This something may be that which causes the degeneration. If so, the smallest amount of this substance would be deposited in the wing tip (the nick) and increasing amounts in the parts which become destroyed by-and-by in the series up to vg. Or the

something might be a stuff necessary for normal differentiation, for example, a growth hormone or a vitamin which enters the wing at its base and slowly spreads all over the wing in a path which is represented by the "moving picture" from vg to nicked. Wherever this vitamin does not penetrate, degeneration and resorption set in. The series of phenotypes would then be the product either of the quantity of the vitamin, sufficient or not sufficient to flow over the entire wing, or of the speed of production of this vitamin relative to the normal growth of the wing bud. The pattern of the scalloping in the vg series would represent the path along which this substance diffuses through the wing area. In a further paper on the genetics of the case we shall again take up this idea.

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GENE AND CHARACTER
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GENE AND CHARACTER

V. FURTHER DATA ON THE *vg* DOMINIGENES IN *DROSOPHILA MELANOGASTER*

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INTRODUCTION

IN THE SECOND PAPER of this series (Goldschmidt, 1935) the following facts were emphasized: (1) The heterozygote of *vg* (vestigial) and wild type, usually of wild type because of the recessiveness of *vg*, becomes more or less intermediate if a group of modifying genes, called dominigenes, are present. These are genes which otherwise do not have any visible effect. (2) There is one main dominigene within the X-chromosome, originally derived from the spineless race, but also present elsewhere. It seemed probable that it is an allele of *cut* (*ct*). (3) The action of this gene in shifting dominance requires the additional presence of two dominant autosomal genes A and B in the second and third chromosomes in at least one quantity each, that is, AaBb. (4) An increase in the number of these genes from AaBb to AABb to AABB increases the effect of dominance as measured by the amount of scalloping of the wings (herein called the *kn* effect). (5) The *ct* allele must be homozygous in the ♀, simplex in the ♂ to produce any effect. (6) AB alone produce a very slight effect of dominance in the *vg*/+ heterozygote, a nick in the wings of about 1 per cent of the flies.

While this former paper was in press a paper was published by Stanley (1935), who independently reached similar conclusions (p. 480). He also found a primary modifier of dominance in the X-chromosome and also reached the conclusion that further modifiers must be present, which he assumed to be contained both in the *vg* and in the + lines. All of them act only, but not always, in *vg*/+. He succeeded in establishing by selection a line almost homozygous for these modifiers.

In my former paper (part II of this series) some points could not be settled satisfactorily: (1) The final proof was missing that the X-dominigene is a *cut* allele. (2) The chromosome containing the gene B was not known. (3) There were some unsatisfactory results concerning lines supposedly homozygous for all dominigenes. The present paper will deal with these matters.

THE CUT ALLELE

The dominigene in the X-chromosome will henceforth be called *ct^{do-vg}*. Females *vg*/+ can show the shifting of dominance (intermediate condition of wings: scalloping or *kn* effect) only if they are homozygous for this gene (of course in the presence of AB). If this dominigene is a *ct* allele it is to be expected that the compound *ct*/*ct^{do-vg}* has at least the same effect as *ct^{do-vg}* homozygous. The combination of this compound with AB was produced as follows:

(1) ♀ kn^* from selected stock homozygous for all three dominigenes and heterozygous for vg were crossed to ct^s ♂. We worked exclusively with the very typical allele ct^s . This is meant henceforth when ct is used.

All ♀ $vg/+$ ct/ct^{do-vg} A/a B/b are kn ; ct completely replaces the dominigene. The gene ct , when homozygous, has an appreciable phenotypic effect, which, though cutting off parts of the wing, follows a pattern very different from the

TABLE 1
F₁ ♀ $vg/+$ HOMOZYGOUS IN ALL DOMINIGENES × ♂ ct

No.	♀	♂	♀ kn	♂ kn	kn class
5800.....	79	56	73	63	I-IV, mostly I
5807.....	57	40	31	36	I-IV
Total.....	136	96	104	99	

vg -series pattern. The kn classes, however, show that in the compound with the dominigene, ct does not act more strongly upon the phenotype than the dominigene, which latter alone has no phenotypic effect whatsoever. All other crosses confirm this fact.

(2) ct flies were crossed with selected stock homozygous for vg , ct^{do-vg} , and A , and heterozygous for B , which is linked with vg (see below).

No. 5799 ♀ ct × ♂ vg sel. 56 ♀ kn 19 ♀ + 41 ♂ ct

No. 5825 ♀ vg sel × ♂ ct . 53 ♀ kn 57 ♂ kn 15 ♀ + 18 ♂ +

Again ct completely replaces ct^{do-vg} in the female. The 3:1 ratio of kn : + proves that the ct stock was heterozygous for B . (A and B are frequently found in different stocks.)

(3) F₂ and backcrosses with 1 and 2.

a. F₂ with kn mothers

TABLE 2
DIFFERENT F₂ FROM CROSSES 1 AND 2 WITH kn MOTHERS

Number	Cross	vg		ct		+		kn		
		♀	♂	♀	♂	♀	♂	♀	♂	
6000	F ₂ 5799 ² ♀ kn ♂ ct	23	12	28	24	22	17	28	28	1 ♂ strap. See below.
6001	F ₂ 5799 ² ♀ kn ♂ ct	20	6	5	6	27	19	30	19	
6002	F ₂ 5799 ² ♀ kn ♂ ct	10	10	30	36	18	20	14	23	
6003	F ₂ 5799 ² ♀ kn ♂ ct	1	1	4	15	13	17	9	24	
6004	F ₂ 5799 ² ♀ kn ♂ ct	11	11	31	21	17	12	17	24	2 ♂ strap. See below.
6005	F ₂ 5799 ² ♀ kn ♂ ct	16	17	9	20	16	14	15	26	

The segregation is 1 vg/vg 2 $vg/+$ 1 $+/+$ in regard to vg . Among these the $+/+$ are $\frac{1}{2}$ ♀ ct/ct $\frac{1}{2}$ ♀ ct/ct^{do-vg} $\frac{1}{2}$ ♂ ct $\frac{1}{2}$ ♂ ct^{do-vg} . Among the $vg/+$ the same combinations occur. This means that $\frac{1}{2}$ are ct , $\frac{1}{2}$ kn , provided that A and B are present. The vg/vg are all vestigial, which are less viable, especially so in

* kn always means some type of scalloping.

the presence of the dominigenes; they therefore appear in smaller numbers than expected. The parents of A and B might have been AABb or AaBb. This allows for different segregations of these genes and therefore different ratios of kn and + (see later analysis of AB). Again we see that ct replaces the dominigene in the female. Simplex in the male, it produces, of course, the cut phenotype.

b. F_2 from + mother

TABLE 3
DIFFERENT F_2 FROM + MOTHERS

Number	Cross	vg		ct		+		kn	
		♀	♂	♀	♂	♀	♂	♀	♂
6006	5299 ² ♀ + ♂ ct.....	5	7	14	15	15	15	11	21
6007	5299 ² ♀ + ♂ ct.....	4	9	23	24	20	40	13	25
6008	5299 ² ♀ + ♂ ct.....	17	10	61	51	22	25	36	31
6009	5299 ² ♀ + ♂ ct.....	9	6	18	13	22	23	31	22

The mothers of these crosses were not different from the foregoing except in being A/a b/b. The only difference to be expected is therefore that the kn class will be relatively smaller. Otherwise we see ct again replacing the dominigene in the female.

The segregation of these two groups together is 448 ct 369 + and kn 205 vg; the expectation is $\frac{3}{8}$ ct $\frac{3}{8}$ + and kn $\frac{2}{8}$ vg, provided that vg/vg ct/ct are phenotypically vestigial.

c. Backcrosses with vg homozygous for dominigenes
ct^{do-vg} A; heterozygous for B

TABLE 4
BACKCROSSES WITH vg HOMOZYGOUS FOR DOMINIGENES ct^{do-vg} A; HETEROZYGOUS FOR B

Number	Cross	vg		ct		+		kn		Further
		♀	♂	♀	♂	♀	♂	♀	♂	
6019	5800 ♀ kn × vg sel.....	34	29	—	8	25	7	37	13	3 ♂ strap
6020	5799 ♀ kn × vg sel.....	33	17	—	13	12	4	35	23	
6021	5800 ♀ + × vg sel.....	—	—	—	37	23	5	85	52	
6022	5800 ♀ + × vg sel.....	—	—	—	29	35	11	60	32	
6042	5800 ♀ + × vg sel.....	—	—	—	17	10	2	92	39	
6041	vg sel × 5799 ♂.....	29	24	—	—	20	11	40	20	

Expectations: Nos. 6019, 6020. $\frac{1}{2}$ vg/vg. $\frac{1}{2}$ vg/+. Of these again $\frac{1}{2}$ ♀ ct/ct^{do-vg} $\frac{1}{2}$ ♀ ct^{do-vg}/ct^{do-vg} ♂ $\frac{1}{2}$ ct $\frac{1}{2}$ ct^{do-vg}, that is, $\frac{1}{2}$ vg ♀ $\frac{1}{4}$ ♀ kn $\frac{1}{8}$ ♂ kn $\frac{1}{8}$ ♂ ct, provided AB are present. If AB segregate as expected, a corresponding number of normals will appear.

Nos. 6021, 6022, 6042. Mother had no vg but was ct/ct^{do-vg}. Therefore daughters are expected to be kn, and sons $\frac{1}{2}$ kn $\frac{1}{2}$ ct. But AB were not homozygous,

and therefore different proportions of normals must segregate. Again *ct* has replaced *ct^{do-vg}* in the compound female. If, however, a male contains *ct*, it will always have the phenotype *cut*, just like a homozygous female, even if *vg/+* *A/a* *B/b* are present. This is proved also by the direct cross *ct* ♀ × *vg* sel. ♂. But there is a possibility that such a male, if homozygous for *AB*, behaves differently and is *kn* instead of *cut*. A decisive experiment with properly marked second and third chromosomes has not yet been performed. The results of the *F*₂ in table 2 are not in favor of such an assumption, as *kn* and *ct* ♂ appear in equal numbers.

Finally, a number of controls were made by introducing many other genes instead of *cut* into similar combinations. None of them had a dominigene effect. If, however, *cut* was introduced in the form of *X-ple* = *sc ec ct v g f*, the same results were obtained as with *ct* alone.

The gene *ct*, then, has the same dominigene effect as its allele *ct^{do-vg}*, though the latter alone has no phenotypic effect at all. We know now that *ct^{do-vg}* heterozygous in a female containing *vg/+* has no effect, or hardly any (Goldschmidt, 1935). If in this combination we replace the dominigene by its allele *ct*, a visible effect is obtained.

TABLE 5

*F*₁ *vg* × *ct*

3594	<i>vg</i> ♀ × <i>ct</i> ♂	120 ♀ 7 ♀ <i>kn</i> class I all ♂ +
3595	<i>ct</i> ♀ × <i>vg</i> ♂	81 ♀ 5 ♀ <i>kn</i> class I ♂ <i>ct</i>

Not less than 6 per cent of the females *vg/+ ct/+* are *kn*; *ct*, which in the compound only equals the action of the lower allele, exercises a dominigene effect of a much higher grade in the combination just described.

The experiments which have been described above, then, together with the localization of the gene, show that *ct^{do-vg}* is actually a lower allele of *ct*. There is still the possibility that the dominigene is another gene closely linked with *ct*. This is not very probable, because it would require on the basis of the actual facts that such a gene be always homozygous if *ct* is homozygous. Only one test for the possibility of such a linkage was made. An *F*₁ ♀ from *cv ct* × + was crossed to a *vg* ♂ homozygous for all dominigenes. If *do-vg* is different from *cut*, it might be separated by crossing over, and individuals would be produced with *cv do-vg*. The ♂ of this constitution would be *kn* in the presence of *AB*. If, however, *do-vg* is an allele of *ct*, crossover *cv* individuals can never be *kn*, which was the true situation. Therefore we may assume that *ct^{do-vg}* is actually a *cut* allele.

Corroboration is finally forthcoming from a different source. In connection with another line of work, a cross between *px* (plexus) and *vg* was obtained in which the *F*₁ individuals were high-grade *kn* (classes VII–VIII). The suspicion was that a higher allele of the dominigene was present in the particular *px* individual. But *F*₁ turned out to be sterile. Meanwhile Mr. Ma, while working with me, obtained this cross once more; a line with this effect could be

started. From this line, however, individuals of the phenotype *ct* suddenly appeared. The analysis has not yet been completed; but it again shows the close connection between *ct* and *do-vg*.

In tables 1 to 5 a few males of the phenotype *strap* are occasionally mentioned. Their appearance is typical for such crosses and very frequently they have, besides, abnormal eyes. They turned out to be homozygous for *vg* and *ct* and their type is inherited in some fashion. A similar phenomenon had been found in certain combinations of *vg* and certain other genes. A special analysis has been begun and therefore the phenomenon will only be mentioned here. It remains to be seen whether the phenomenon is connected with Harnly's dimorphic type of *vg* (Harnly, 1935).

THE AUTOSOMAL DOMINIGENES

In a former paper (part II), the two autosomal dominigenes A, B acting together with the *ct* allele were described, and it was stated that a homozygous line had been established, segregating as if *vg/+* were intermediate. (Stanley, 1935, established a similar line, it seems.) In my former paper (1935, part II, p. 86) I added, however, that there were some complications, which would be taken up later. These will now be considered.

LINKAGE OF B WITH *vg*

The complications just mentioned are the following. (1) Very frequently in such a segregating line different ratios are obtained for females and males, namely, too few females in the *kn* class and too many in the wild-type class. (2) A homozygous strain for all dominigenes is not easily established.

This second difficulty has a simple cause. In the former paper (1935, part II), it was stated that the dominigene A is situated within the third chromosome, not far from spineless. No definite location was assigned to B. If B is situated in the second chromosome not far from *vg*, a homozygous line for B and *vg* can be obtained from a cross *vg b* × + B only if crossover between *vg* and B occurs. The nearer the two loci, the more difficult to establish such a line by selection, B not having any visible effect alone. As a matter of fact, most selections of *kn* individuals heterozygous for *vg*, homozygous for *ct^{do-vg}*, and probably for A, did not give a completely homozygous line for A and B. Such lines, if bred from *kn* individuals, segregated into +, *kn*, and *vg*, but not in a 1:2:1 ratio. Extracted *vg* × extracted wild type from such a line did not produce 100 per cent *kn*, but *kn* and normals. It was to be assumed, therefore, that B at least had not become homozygous. However, after repeating these selections with many pairs, the homozygous lines were finally obtained, presumably from crossover individuals. Here, then, all individuals are homozygous for the three dominigenes, whether they are *vg/vg*, *vg/+*, or *+/+*, and each class contains as many females as males. But if we breed in such a line from the *kn* individuals, which are always *vg/+*, there appears not the expected ratio of 1+:2 *kn*:1 *vg*; instead, there are too many *kn* and too few *vg* and +. In other words, both homozygous *vg/vg* and *+/+* in combination with all the dominigenes are less viable than the similar heterozygous *vg/+*. These

homozygous lines hereafter remained constant, always showing the same segregation, but frequently being rather weak, with much sterility. Table 6 contains the data of such segregations in four generations of the combination $kn \times kn = vg \ A \ B \ ct^{do-vg} / + \ A \ B \ ct^{do-vg} \times dto$.

TABLE 6
SEGREGATION IN FOUR GENERATIONS OF $kn \times kn$ BREEDING IN THE SELECTED LINE

Number	vg		kn		+	
	♀	♂	♀	♂	♀	♂
5906.....	13	11	38	36	12	8
5914.....	9	16	26	34	15	9
5953.....	6	12	26	25	15	6
6085.....	7	6	11	14	5	1
6092.....	3	—	8	4	4	2
6273.....	5	7	37	32	9	14
6300.....	3	4	7	9	2	3
6682.....	7	5	28	41	10	12
6683.....	6	6	27	32	11	8
6684.....	3	3	11	15	4	2
6689.....	4	3	16	26	14	9
6692.....	—	2	18	22	6	4
6693.....	3	3	17	20	4	6
6694.....	5	4	22	27	15	13
6695.....	2	7	30	27	9	8
6696.....	1	7	13	17	4	4
6794.....	14	9	28	28	19	5
6799.....	21	13	29	31	18	9
Total.....	112	118	392	440	176	123

If the extracted *vg* flies from such a line are crossed to extracted wild type, 100 per cent *kn* ought to be obtained. Strangely enough, this is not completely the situation, as the following crosses show.

TABLE 7
EXTRACTED *vg* \times EXTRACTED +

6312	Extr. <i>vg</i> \times extr. +.....	63 ♀ 55 ♂ <i>kn</i> 9 ♀ +
6793	Extr. <i>vg</i> \times extr. +.....	30 ♀ 49 ♂ <i>kn</i> 4 ♀ 1 ♂ +
5949	Extr. <i>vg</i> \times extr. +.....	29 ♀ 35 ♂ <i>kn</i> 1 ♀ +
	Total.....	122 ♀ 139 ♂ <i>kn</i> 14 ♀ 1 ♂ +

There were 5.4 per cent normal exceptions, mostly females. We shall learn at once that the shifting effect of the dominigenes is smaller in females; we know, further, that the effect is susceptible to modifying influences. Therefore we are inclined to explain the exceptions by these two observations. If this be the situation, the normal exceptions ought to behave genetically like

their *kn* sisters, that is, give the $-1 : +2 : -1$ ratio if bred to a *kn* brother. The result of testing four exceptional ♀ is shown in table 8.

This agrees with the expectation (compare table 6).

The experiments thus show: (a) *B* is linked with *vg*. (b) *ct^{do}-vg* *AB*, when homozygous, have a sublethal effect on flies not heterozygous for *vg*. (c) Such homozygous lines are best bred from *kn* individuals (being *vg*/+); they segregate into $<1 \text{ } vg : >2 \text{ } kn : <1 \text{ } wild$.

TABLE 8
EXCEPTIONAL ♀ × *kn* BROTHER

Number	<i>vg</i>		<i>kn</i>		+	
	♀	♂	♀	♂	♀	♂
6685.....	—	—	7	9	1	1
6686.....	4	4	25	21	11	6
6687.....	—	—	10	11	2	2
6688.....	—	—	20	11	4	2
	—	—	—	—	—	—
Total.....	4	4	62	62	18	11

DIFFERENTIAL EFFECT OF THE DOMINIGENES UPON THE PHENOTYPE OF ♀ AND ♂

If *kn* ♀ and ♂ from lines selected for dominigenes, but not yet homozygous in both autosomal dominigenes, are bred together, a certain proportion of normals will appear among the offspring, presumably of the constitution *AAbb* (*A*, being not linked with *vg*, is easily obtained homozygous). In such broods it was found frequently that the *kn* class contained fewer ♀ than ♂, and that simultaneously the females missing in the *kn* class were added to the wild-type class. The most obvious explanation is that certain combinations of dominigenes act only upon the male. As *ct^{do}-vg* must always be homozygous (duplex in the ♀, simplex in the ♂), if the shifting effect occurs, it might be assumed, for example, that *AABB*, *AABb*, *AaBB*, *AaBb* produce the *kn* effect in both ♀ and ♂, but that the combination *AAbb* makes only the males (or part of the males) intermediate (*kn*).

From the foregoing analysis it follows that in a selected line (selection of *kn* = *vg*/+ and dominigenes) which has not become completely homozygous, probably only the gene *B*, linked with *vg*, is still heterozygous, excepting rare crossover individuals. Breeding from such *kn* individuals and accepting the tentative view that *AAbb* ♂ are *kn* and ♀ are not, the following possibilities are given:

♀ *kn* may be: *AABB* (c.o.), *AABb*.

♂ *kn* may be: *AABB* (c.o.), *AABb* and *AAbb*.

Random matings of *kn* ♀ ♂ homozygous for the other two dominigenes may be:

1. *BB* × *BP*. All *vg*/+ offspring *kn*, segregation as described $<1 \text{ } vg : >2 \text{ } kn : <1 \text{ } wild$.

TABLE 9
BREEDING OF kn \times kn IN SELECTED LINE

1. Presumably BB \times BB or Bb			
5906.....	13 ♀ 11 ♂ vg	38 ♀ 36 ♂ kn	12 ♀ 8 ♂ wild
5914.....	9 ♀ 16 ♂ vg	26 ♀ 34 ♂ kn	15 ♀ 9 ♂ wild
5933.....	6 ♀ 12 ♂ vg	26 ♀ 25 ♂ kn	15 ♀ 6 ♂ wild
Total.....	28 ♀ 39 ♂ vg	90 ♀ 95 ♂ kn	42 ♀ 23 ♂ wild
Exp.....	< 1	> 2	< 1

2. Presumably BB ♀ \times bb ♂			
5912.....	3 ♀ 4 ♂ vg	10 ♀ 15 ♂ kn	4 ♀ 6 ♂ wild
6085.....	7 ♀ 6 ♂ vg	11 ♀ 14 ♂ kn	5 ♀ 1 ♂ wild
4275.....	65 ♀ 59 ♂ vg	124 ♀ 138 ♂ kn	70 ♀ 58 ♂ wild
5220.....	4 ♀ 3 ♂ vg	14 ♀ 14 ♂ kn	8 ♀ 5 ♂ wild
H 12.....	21 ♀ 14 ♂ vg	31 ♀ 36 ♂ kn	20 ♀ 15 ♂ wild
Total.....	100 ♀ 86 ♂ vg	190 ♀ 217 ♂ kn	107 ♀ 85 ♂ wild
Exp.....	1	2	1

3. Presumably Bb \times Bb			
4092.....	14 ♀ 16 ♂ vg	47 ♀ 48 ♂ kn	43 ♀ 22 ♂ wild
4277.....	24 ♀ 23 ♂ vg	46 ♀ 70 ♂ kn	44 ♀ 26 ♂ wild
H 8.....	27 ♀ 19 ♂ vg	31 ♀ 41 ♂ kn	20 ♀ 10 ♂ wild
H 11.....	24 ♀ 31 ♂ vg	38 ♀ 45 ♂ kn	33 ♀ 27 ♂ wild
6271.....	4 ♀ 3 ♀ vg	19 ♀ 31 ♂ kn	25 ♀ 16 ♂ wild
Total.....	93 ♀ 92 ♂ vg	181 ♀ 235 ♂ kn	165 ♀ 101 ♂ wild
Exp.....	2/8 2/8	3/8 4/8	3/8 2/8

4. Presumably Bb \times bb			
5905.....	8 ♀ 11 ♂ vg	25 ♀ 33 ♂ kn	36 ♀ 13 ♂ wild
6075.....	18 ♀ 12 ♂ vg	22 ♀ 36 ♂ kn	41 ♀ 24 ♂ wild
6076.....	18 ♀ 29 ♂ vg	23 ♀ 42 ♂ kn	59 ♀ 38 ♂ wild
4465.....	12 ♀ 7 ♂ vg	14 ♀ 22 ♂ kn	18 ♀ 15 ♂ wild
5219.....	8 ♀ 11 ♂ vg	14 ♀ 20 ♂ kn	27 ♀ 11 ♂ wild
Total.....	64 ♀ 70 ♂ vg	98 ♀ 153 ♂ kn	181 ♀ 101 ♂ wild
Exp.....	1/4 1/4	1/4 1/2	1/2 1/4

2. $Bb \times Bb$. Similar result. But as only the completely homozygous condition of AB is sublethal for the vg and $+$ segregates, the ratio will be nearer to 1:2:1.

3. $Bb \times Bb$. $\frac{1}{4}$ bb segregate. Within the $vg/+$ class these are normal if ♀ , kn (or in part kn) if ♂ . The segregation is about:

♂ : 1 vg : 2 kn : 1 $+$;
 ♀ : $\frac{2}{8}$ vg : $\frac{3}{8}$ kn : $\frac{3}{8}$ $+$.

4. $Bb \times bb$. All offspring Bb ; segregation exactly 1 vg : 2 kn : 1 $+$.

5. $Bb \times bb$ ♂ . ♂ segregate 1:2:1. Half of the ♀ are bb , that is, wild type; therefore ♀ segregate as 1 vg : 1 kn : 2 $+$.

TABLE 10
UNEXPECTED RESULTS FROM BROODS AS IN TABLE 9

Number	vg		kn		$+$	
	♀	♂	♀	♂	♀	♂
5908.....	6	10	9	14	36	27
6078.....	5	6	6	10	23	18
6080.....	4	9	6	17	25	18
6082.....	13	15	17	17	44	32
H 92.....	11	11	4	8	25	26
H 288.....	18	12	20	35	74	53
H 289.....	26	28	35	44	93	87
H 286.....	17	24	36	58	51	71
6303.....	15	16	24	36	73	40
6316.....	9	6	4	16	37	29
Total.....	134	137	161	255	481	413
Possibly.....	<1	<1	<1	1	2	2

If not all bb ♂ exhibit the kn phenomenon, but only a part of them, the male classes will be somewhat shifted toward the type of female segregation.

Many crosses of this type have been made and all the results just tabulated did occur, as table 9 shows.

These results agree with the interpretation which was tentatively derived from them. But besides these typical results a number of crosses were obtained which gave a quite typical, but different, result. The first group is shown in table 10.

If we compare table 10 with table 9, group 4, we realize that the females show about the same ratios and that the males here follow the females. (It has to be kept in mind that vg always segregates as less than $\frac{1}{4}$ of the total.) This would mean that here only a part of the bb ♂ were kn , the rest being wild type like the females. If we calculate from the results of group 4 in table 9 how many bb ♂ must have been kn or normal, we find that most of them (about 80 per cent) were kn (expectation for all bb normal, 169.3 kn : 84.7 $+$; found, 153:101). In the group of crosses which we are now discussing, however, only

6. The shift of dominance takes place in the female if at least A and B are present in simplex form (AaBb). Males of the constitution AAbb are mostly kn, whereas females are always normal.

7. The intensity of the effect, measured by the amount of scalloping (kn class), is proportional to the number of the autosomal dominants, that is, maximal for AABB.

8. The autosomal dominigenes alone have hardly any phenotypic effect. If present in vg/+ individuals and in the absence of the ct allele, they produce just the inception of the nick effect upon 1 per cent of the individuals.

9. Individuals homozygous for all three dominigenes are weaker and frequently sterile. This applies especially to vg/vg and +/+ individuals; much less so to vg/+ heterozygotes. Lines bred from kn individuals (vg/+) homozygous in all dominigenes segregate, therefore, as: few vg; many intermediates (kn); few wild type.

10. There are genes known to be antagonistic to the dominigene action; for example, in the quintuple line.

11. The dominigenes may be isolated from many *Drosophila* lines. The ct allele was found most frequently in the ss line; the others seem to be ubiquitous.

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GENE AND CHARACTER
VI. DOMINIGENES AND v_g ALLELOMORPHS

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VI. DOMINIGENES AND *vg* ALLELOMORPHS

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IN TWO FORMER PAPERS, parts II and V, by the senior author (Goldschmidt, 1935, 1937) the genetics of the so-called dominigenes for the expression of the vestigial character in the heterozygote were described. Three genes collaborate in shifting the phenotype of *vg/+* from normal toward a nicked or notched intermediate: (1) *ct^{do-vg}*, a cut allele in the first chromosome, which is without phenotypic effect alone; (2) a gene A in the third chromosome; (3) a gene B in the second chromosome. The shifting toward dominance takes place only if *ct^{do-vg}* is homozygous (one dose only in the male) and if also at least one dose of A and B is present. But some of the AΔbb males also show the effect. The amount of the effect is proportional to the number of these genes, increasing from AaBb to AABB, phenotypically represented by classes I-V of notching. It was further shown that the same effect could be produced by proper temperature action, which can be superimposed also on the dominigene effect. It is further known that certain of the *vg* alleles give, either in homozygous or in heterozygous condition, the same phenotypic effect in an orderly way (Mohr, 1932). If we want to derive a theoretical understanding of the entire series of facts in terms of gene action, the question arises how these dominigenes act if combined with the different *vg* alleles instead of *vg*. The following experiments will serve to answer this question.

The *vg* alleles which we used were *vgⁿⁱ* = nicked, *vg^{no}* = notched, and *vg^{Nw}* = no-wing. All were obtained through the courtesy of Professor O. L. Mohr, of Oslo. When we bred these stocks, we realized that they behaved differently from what the original data of Mohr led us to expect. We understand that Mohr also had noticed the change of phenotypic expression of the notching in some of his lines, probably the result of modifying factors. We do not intend to go into the details of this problem; for our purposes the only important point is that the lines showed a constant behavior. Table 1 serves to compare our data with Mohr's results. Of course, the interpretation of our experiments must be based upon the data of this table, which represents the typical behavior in our cultures as controls. The fact ought to be emphasized (see table 1) that some of the alleles produce no phenotypic effect at all in homozygous condition and that in certain homozygous or heterozygous combinations only a certain percentage of the individuals are visibly changed. As in our previous papers, the amount of scalloping of the wings is expressed in terms of classes I-XII, I meaning a nick in one wing and XII no-wing.

Table 1 shows that in all our controls the percentages, as well as the classes, of kn individuals are much higher than in Mohr's original crosses. This might be a result of an accumulation of modifying factors or of a difference in en-

vironment. However this may be, all comparisons with the experiments to be described will have to be made on the basis of the numbers in our controls. In one of the combinations (no \times ni) reciprocal crosses gave markedly different results; a sex-linked modifier might have brought this about.

TABLE 1
THE kn PHENOMENON IN THE CONTROLS

Constitution	Controls				Mohr's lines		Remarks
	+	kn*	class kn	Percentkn	Percentkn	Class kn	
$\frac{vg^{n1}}{vg^{n1}}$	491	14	I	2.8	0	—	$\frac{vg^{n1}}{+}$ all normal
$\frac{vg^{n1}}{vg}$	16	433	I-III	96.4	27, 1	I-II	
$\frac{vg^{n1}}{vg^{nw}}$	—	349	I-IV	100†	70, 7	III-IV	
$\frac{vg^{no}}{vg^{no}}$	106	3003	I-IV	96.6	42, 4	II-III	
$\frac{vg^{no}}{vg^{n1}}$ (no \times ni)	195	103	I-II	34.6	0, 2	I-II	
$\frac{vg^{no}}{vg^{n1}}$ (ni \times no)	455	49	I-II	9.7	0, 2	I-II	
$\frac{vg^{no}}{+}$	all	—	—	—	—	—	
$\frac{vg^{no}}{vg}$	—	1051	VIII-IX	100	100	VI-VII	
$\frac{vg^{no}}{vg^{nw}}$	—	281	IX-XI	100‡	100	VIII	
$\frac{vg^{nw}}{vg^{nw}}$	lethal						
$\frac{vg^{nw}}{+}$	21	5	I	18.8	1, 3	I-II	
$\frac{vg^{nw}}{vg}$	—	180	X-XI	100	100	Stumps	

* kn is the general abbreviation for all types of nicking, notching, and scalloping.

† No wing is homozygous lethal; F₁ with it therefore segregates 50 per cent normals. The actual percentage was 50.1.

‡ Like 100†. Actual percentage of normals, 51.8.

To analyze the action of the three dominigenes when combined with the different vg alleles, these alleles and their compounds were crossed with a line homozygous for all dominigenes, the derivation of which was described in a former paper. F₁ females are heterozygous for the dominigene in the first chromosome and males may contain it if it is carried by the mother. All contain A and B at least in one dose, a condition which allows for the dominigene effect. F₂ of such a cross produces one-half females homozygous for *et^{do}-vg* and

with different combinations of A and B. Of course, backcrossing with the dominigene line in both directions produces homozygous females and eliminates the possibility of segregation of flies without A or B or both. F_1 then informs us of the minimum action of the dominigenes in shifting the phenotype of the heterozygote of the respective allele. F_2 and backcrosses demonstrate the range of action (all three dominigenes \pm homozygous) upon the heterozygotes as well as upon the homozygotes in regard to the *vg* alleles. The homozygous line of dominigenes, otherwise wild type, will be abbreviated Doho (see table 2).

TABLE 2
PHENOTYPES OF THE NICKED COMBINATIONS; HETEROZYGOUS

Generation	Cross	+		kn		class		kn per cent	
		♀	♂	♀	♂	♀	♂		
F_1	$\text{Doho} \times \frac{\text{vg}^{n1}}{\text{vg}^{n1}} \dots\dots$	224	201	1	1	I	I	0.4	The vg^{n1} line already contained some dominigenes. Therefore ♀ = ♂
F_1	$\text{Doho} \times \frac{\text{vg}^{n1}}{\text{vg}^{n1}} \dots\dots$	195	218	1	2	I	I	0.7	
RF_2	$\frac{\text{vg}^{n1}}{+} \times \text{Doho} \dots\dots$	496	436	7	8	I	I	1.5	
RF_3	$\text{Doho} \times \frac{\text{vg}^{n1}}{+} (\text{kn})$	170	143	—	1	—	I	0.3	
RF_4	$\text{Doho} \times \frac{\text{vg}^{n1}}{+} (\text{kn})$	244	234	2	4	I	I	1.8	
RF_9	$\text{Doho} \times \frac{\text{vg}^{n1}}{\text{vg}^{n1}} (\text{sel.})$	186	169	2	3	I	I	1.4	
RF_{10}	$\text{Doho} \times \frac{\text{vg}^{n1}}{\text{vg}^{n1}} \dots\dots$	171	144	—	4	—	I	1.4	
RF_{11}	$\text{Doho} \times \frac{\text{vg}^{n1}}{\text{vg}^{n1}} \dots\dots$	130	101	1	4	I	I	2.1	

DOMINIGENES ADDED TO $\text{vg}^{n1}/+$, WHICH OTHERWISE WOULD BE
PHENOTYPICALLY WILD TYPE

Only the two F_1 crosses are heterozygous for vg^{n1} and for A and B. The crosses RF_{9-11} are heterozygous for vg^{n1} but probably homozygous for all dominigenes, because they were taken from the selected lines to be described in the next paragraph. RF_{2-4} segregate into $\frac{1}{2} + \frac{1}{2} \text{vg}^{n1}/+$, but they are more or less homozygous for the dominigenes because they have been repeatedly backcrossed to Doho. If we analyze the results of table 2 we find:

- (1) $\text{vg}^{n1}/+ \text{ct}^{\text{Do-vg}} \text{A/a B/b} = 0.6$ per cent kn;
- (2) $\text{vg}^{n1}/+ \text{ct}^{\text{Do-vg}}/\text{ct}^{\text{Do-vg}} \text{A/A B/B} = 2$ per cent kn.

The addition of the dominigenes to the heterozygote $\text{vg}^{n1}/+$ produces a shifting of the dominance, which increases with the number of dominigenes.

DOMINIGENES ADDED TO vg^{n1}/vg^{n1} , WHICH IN OUR CONTROLS
THREW 2.8 PER CENT OF kn

The procedure was to breed from the foregoing backcross RF_4 , which was presumably homozygous for the dominigenes, using kn individuals, which must have been heterozygous for vg^{n1} . Selection of kn in this and the next generation ought to establish a true breeding line homozygous in both the vestigial allele and the dominigenes. As a matter of fact, the presence of a large number of kn in the first two selected generations (12.3 and 16.7 per cent) indicated the presence of $\frac{1}{4}$ homozygotes plus dominigene effect. In the following generation the homozygous line was established, as table 3 indicates.

TABLE 3
PHENOTYPES OF THE NICKED COMBINATIONS: HOMOZYGOUS

Generation	Cross	+		kn ♀ class			kn ♂ class			kn per cent
		♀	♂	I	II	III	I	II	III	
F_5	$(RF_4)^2$	504	426	37	3	—	50	14	—	12.3
F_6	$F_5 kn$	535	433	52	7	—	104	36	—	16.7
F_7	$F_6 kn$	105	74	19	23	—	45	56	—	43.2
F_8	$F_7 kn$	51	20	25	11	—	24	47	—	60.1
F_9	$F_8 kn$	139	82	58	35	—	92	97	—	56.1
F_{10}	$F_9 kn$	205	82	102	25	—	119	82	—	52.4
F_{11}	$F_{10} kn$	292	115	225	132	1	167	293	8	67.6
F_{12}	$F_{11} kn$	163	52	118	73	—	122	144	1	68.2

The addition of the dominigenes to the homozygote vg^{n1} shifts dominance appreciably. The controls showed 2.8 per cent kn individuals of class I. F_7 - F_{12} of table 3 contain 46.6 per cent ♀ kn , 75 per cent ♂ kn , with a very large number in classes II and III; the latter effect is more pronounced in the male.

DOMINIGENES ADDED TO $vg^{n0}/+$, WHICH IN THE
CONTROLS IS WILD TYPE

Table 4 gives the results for F_1 $Doho \times vg^{n0}/vg^{n0}$ and for backcrosses. Wherever in F_1 the mother was $Doho$, all sons received ct^{do-vg} and the dominigenes, whereas the daughters were heterozygous for this gene in the first chromosome. Such females would be normal with the constitution $vg/+$. But here a good percentage of kn individuals appears in F_1 ; besides, about 25 per cent of the males are kn instead of wild type. The backcrosses RF_2 and RF_4 ought to produce males and females of the same genetic constitution, that is, pure for ct^{do-vg} . But though the percentage of kn males remains the same, the percentage of kn females is not increased to the same number. There is, then, a differential effect for both sexes.

The backcrosses RF_7 and RF_8 were made with extracted homozygotes which were also homozygous for the ct allele.

TABLE 4
PHENOTYPES OF THE NOTCHED COMBINATIONS; HETEROZYGOUS

Generation	Cross	+		kn		kn class		kn per cent		Remarks
		♀	♂	♀	♂	♀	♂	♀	♂	
F ₁	Doho × $\frac{vg^{ao}}{vg^{ao}}$	391	268	18	106	I	I-II	4.4	28.3	
F ₁	Doho × $\frac{vg^{ao}}{vg^{ao}}$	620	416	21	122	I	I-II	3.3	22.7	
RF ₂	Doho × $\frac{vg^{ao}}{+}$	254	233	12	33	I	I-II	9.0	24.8	Per cent calcul. for $\frac{vg^{ao}}{+}$
RF ₂	Doho × RF ₁ kn	355	314	18	56	I-II	I-II	9.7	30.3	Per cent calcul. for $\frac{vg^{ao}}{+}$
RF ₄	RF ₂ kn × Doho	73	73	6	13	I-II	I-II	15.0	30.0	Per cent calcul. for $\frac{vg^{ao}}{+}$
RF ₇ =F ₁	F ₄ kn × Doho	466	299	26	110	I-II	I-II	5.3	26.6	Mother $\frac{vg^{ao}}{vg^{ao}}$
RF ₈ =F ₁	Doho × F ₄ kn	527	396	23	64	I-II	I-II	4.2	27.8	Father $\frac{vg^{ao}}{vg^{ao}}$

It results that the dominigenes shift the phenotype of $vg^{no}/+$ from wild type toward the type of the homozygote vg^{no}/vg^{no} . This effect is much greater in the male than in the female, namely, 22.4 and 6.1 per cent, respectively. Also, females heterozygous for ct^{do-vg} show a 3.7 per cent effect.

DOMINIGENES ADDED TO vg^{no}/vg^{no} , WHICH IN THE CONTROLS
THROWS 96.6 PER CENT OF kn INDIVIDUALS, CLASSES I—II

This combination was obtained in the same way as the parallel combination for vg^{nl} , by crossing Doho \times vg^{no} and selecting kn individuals in further generations until constancy was attained. This was the situation in F_4 . Twelve

TABLE 5
PHENOTYPES OF THE NOTCHED COMBINATIONS; HOMOZYGOUS

kn ♀ class						kn ♂ class			
I	II	III	IV	V	VI	I	II	III	IV
—	11	1066	133	4	1	—	12	991	94

cultures of succeeding generations gave exclusively kn individuals, as shown in table 5. The dominigenes then shifted the phenotype from 96.6 to 100 per cent kn and simultaneously the grade of scalloping was noticeably increased.

DOMINIGENES ADDED TO $vg^{Nw}/+$, WHICH IN THE CONTROLS
THROWS 18.8 PER CENT OF kn , CLASS I

Whereas no-wing is a more extreme allele than vg , and whereas $vg/+$ with the dominigenes gives 100 per cent kn , the same effect is to be expected at least in this group. Doho was crossed with vg/S Cy; the expectation is normal daughters (or a small percentage of kn as in the vg allele) and $\frac{1}{2}$ ♂ kn . This expect-

TABLE 6
PHENOTYPES OF THE NO-WING COMBINATIONS

Generation	Cross	+		kn ♀				kn ♂			kn per cent
		♀	♂	I	II	III	IV	I	II	III	
F_1	Doho \times $\frac{vg^{Nw}}{S}$ Cy	141	71	4	—	—	—	16	66	2	29.3
RF_2	Doho \times F_1 kn	250	197	52	110	16	—	12	129	39	44.3
RF_3	Doho \times F_2 kn	113	109	31	109	1	—	1	59	72	55.2
RF_4	Doho \times F_3 kn	230	211	26	231	23	1	2	68	157	53.5
RF_5	Doho \times F_4 kn	136	78	14	87	39	—	1	35	94	55.9
RF_6	Doho \times F_5 kn	266	259	57	185	2	—	1	51	164	46.7
RF_7	F_6 kn \times Doho	46	36	10	42	1	—	1	18	13	50.9
RF_8	F_7 kn \times Doho	105	108	21	65	2	—	—	83	21	47.4
RF_9	Doho \times F_8 kn	196	166	35	154	10	1	1	131	50	51.3

TABLE 7
PHENOTYPES OF THE COMPOUNDS PLUS DOMINIGENES

Compound cross	+		kn ♀						kn ♂				kn per cent control	
	♀	♂	I	II	III	IV	IX	I	II	III	IV	IX		
ni × no.....	—	1	3	66	20	—	—	21	38	12	—	—	99.4	36.6
ni × no.....	9	—	24	71	—	—	—	7	47	19	—	—	94.9	36.6
no × ni.....	1	1	7	50	26	—	—	2	63	26	—	—	98.9	9.7
no × ni.....	3	4	7	57	3	—	—	33	46	9	—	—	95.7	9.7
ni × vg.....	—	—	—	—	15	17	—	—	—	21	—	—	100	96.4
vg × ni.....	—	—	—	—	59	6	—	—	4	49	1	—	100	96.4
vg × ni.....	—	—	—	1	52	4	—	—	—	30	37	—	100	96.4
ni × Nw.....	72	74	1	39	20	3	—	2	26	31	3	—	100	100
Nw × ni.....	52	39	44	9	2	—	—	5	21	23	2	—	100	100
no × Nw.....	63	18	19	8	—	—	58	23	16	—	—	72	100	*
no × Nw.....	28	14	5	—	—	—	34	17	2	—	—	25	100	100
Nw × no.....	41	16	21	12	—	—	60	15	25	—	—	60	100	100
no × vg.....	—	—	—	—	—	—	82	—	—	—	—	70	100	100
vg × no.....	—	—	—	—	—	—	(cl. VIII-X) 86 (dto)	—	—	—	—	(cl. VII-X) 75	100	100

* The lower kn classes and the normals belong to 1/3 no/+, the group class IX, to the half Nw/no.

tation is fulfilled, as table 6 shows. A kn ♂ backcrossed to Doho must produce $\frac{1}{2}$ kn of both sexes, and the same is true in all further backcrosses.

If we consider the kn classes in table 6, the effect is of the same type as in similar vg/+ combinations. Homozygosity of A and B does not seem to have been accomplished, as no higher classes appear. It is therefore impossible to tell whether in this combination the shift produced by the dominigenes is more extreme than in the vg combination.

DOMINIGENES ADDED TO DIFFERENT COMPOUNDS

It now remains to test the action of the dominigenes upon different compounds of the vg alleles. To build up these combinations, lines were used which had been made homozygous for the alleles in question as well as for the dominigenes (these lines have been presented in tables 1-6). Crossing produced compounds with the dominigenes of the first and third chromosome certainly homozygous, but the gene B in the second chromosome possibly heterozygous. (To make it homozygous, crossovers must have been selected by chance.) Table 7 presents the results. In the first column, nicked is written ni instead of vg^{n1} , and notched and no-wing are represented in like manner.

The results parallel those previously described. Wherever a compound without dominigenes (control) throws normals and kn, the dominigenes shift the phenotype noticeably in the direction of kn individuals. The class of kn is, however, very little influenced. In the ni-no compound the average class value is a little higher than in the controls, but in all other compounds it is not.

DISCUSSION

The effect of the lower vg alleles belongs to the group of genic effects which become visible only in certain percentages of individuals, or even, under identical conditions, in none of them. (All data in this paper were obtained under constant conditions at 25° C.) There is, as worked out first by Mohr, an orderly series of these percentages in different alleles and their compounds, beginning with no visible effect and progressing to a combination with 100 per cent of all individuals affected. Up to this point the effect increases with respect to percentage as well as to the amount of abnormality of the wing; from this point on, of course, only the intensity of the effect is increased. Timoféeff-Ressowsky has proposed calling the percentage of affected individuals the penetrance of the gene. Of course, in many combinations such an effect might be produced by segregation of modifying genes; in others, like the vg combination, however, it might be caused by definite embryological conditions with which the gene or allele has to work. Only if this can be proved, as with vg where the proof is derived from orderly behavior in the series, might the term penetrance be applied for descriptive purposes. Even here it might well be dispensed with. It is obvious that such an effect which exhibits an orderly quantitative behavior in a series of genic combinations might give some insight into the system of events which connect the gene with the phenotypic effect or, expressed differently, an insight into the methods by which genes control the development of characters. This possibility is increased if, as in this instance,

the actual facts regarding the development of the traits are known. The decisive points are: The development of the wing takes place first in a normal way. But at a definite stage of development parts of the wing anlage become degenerate and are resorbed. If this happens late in development after pupation, the lower classes of the kn effect result; if it happens earlier and on larger areas, the medium type of effect (classes III–V) results; and if it happens

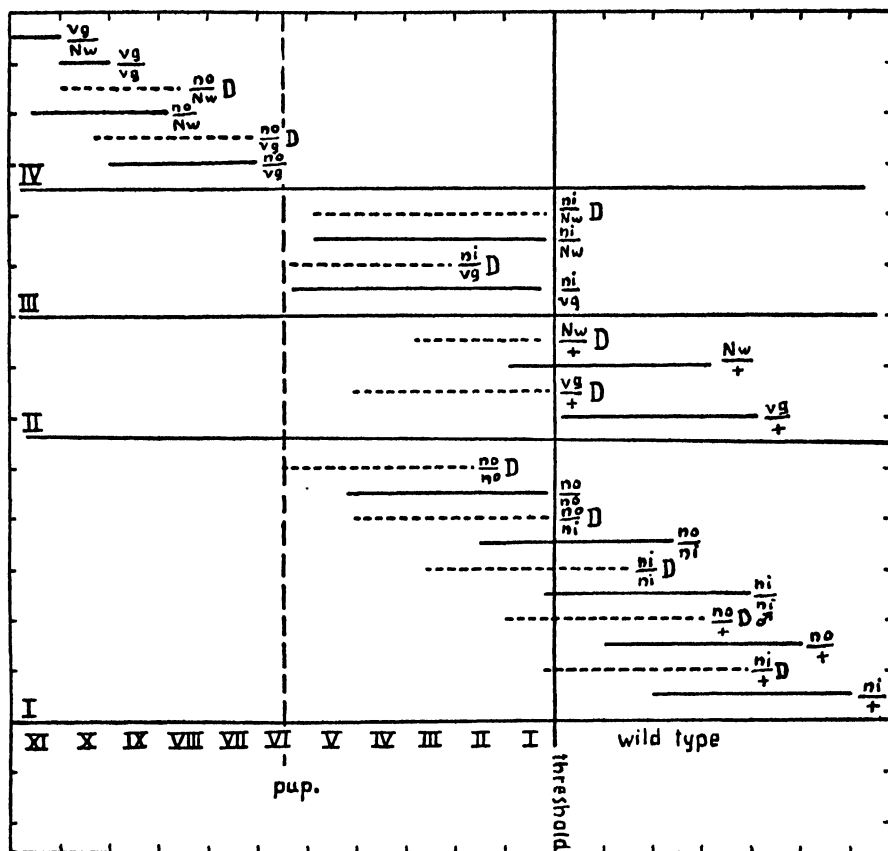


Fig. 1

before pupation and still earlier, the higher types (VI–XI = no-wing) appear. The embryological meaning of the facts has been discussed elsewhere (Goldschmidt, part IV). Here we must try to bring them in line with the genetical facts in order to form a hypothesis which explains the whole problem.

Let us first arrange the facts in a simple diagram (fig. 1). This diagram is based upon the following considerations: (1) Different genic combinations produce different percentages of kn and normals in orderly progression. It is to be assumed, therefore, that the magnitude of the effect of the genes or compounds varies within a certain range, which in the diagram is represented by the length of the respective lines (instead of curves of variation, to simplify the picture). Within this range of effects upon wing development there is a

point beyond which development is normal, and this point is shifted in the different combinations from zero (all normals) to 100 (all kn). We may represent this threshold point beyond which no resorption of the wing epithelium and consequent scalloping takes place as a definite time in development, represented in the diagram by the line marked "threshold." If development proceeds from left to right and the diagram represents the time of the process causing the scalloping, all individuals to the left of this line will be scalloped, and those to the right will be normal. Thus the lines representing the range of variation of different genic combinations also show the respective percentages of scalloping (kn) as taken from tables 1-7. With a shifting of these lines from right to left the number of normals beyond the threshold decreases.

(2) We know that scalloping increases in an orderly way within the series of genic combinations from a nick (class I) to no-wing (class XI). This increase is a very gradual one, beginning near the threshold with the destruction of a single, or of two, marginal hairs. We further know from embryological studies that the higher the degree of scalloping the earlier the onset of resorption of the epithelium. It is therefore to be expected that if the view given in the preceding paragraph is true, the kn classes must increase with the shifting of the range of variation to the left of the threshold line. As a matter of fact, occasional kn individuals in an otherwise normal fraternity are always of class I, and with the increase of the percentage of kn the class value also increases. The actual classes are marked on the base line of the diagram, which now represents for each combination the percentage as well as the kn class found in the experiments.

Let us consider first only the lower section (I) of the diagram containing all combinations of vg^{no} , vg^{nl} , and +, all with and without dominigenes. We are indeed struck by the perfect regularity of the picture: the continuous shift of the range of variation toward the left with higher combinations of the genes, and the perfectly parallel action of the dominigenes (broken lines), shifting each line to the left, that is, increasing the percentages and the class values of the effect. *There can be no doubt that such an orderly result is only possible if the whole phenomenon is caused by a rather simple process with an orderly quantitative variation in time.*

We shall now consider the upper half of the diagram. There is first the heterozygote $vg/+$ with and without dominigenes, which was thoroughly analyzed in two former papers. The dominigene effect is much larger, as in the former combinations, namely, from 100 per cent normals to 100 per cent kn (if all dominigenes are homozygous, and leaving out of account a small percentage of normal females). Above this group we find the corresponding combinations for vg^{Nw} . We notice that in the combination with the dominigenes only classes I-III appear (100 per cent). But here the dominigenes B have not been made homozygous as in the vg combination. A similar combination for vg would give about the same variation. Probably, therefore, vg and Nw behave alike. Comparing these results with the first group of combinations, we find similar behavior, with the difference that the shifting effect of the dominigenes is increased if vg or Nw are present.

We shall consider now the third group in the diagram, containing the compounds of vg^{ni} with vg and vg^{Nw} . The striking fact is that here, where the compounds are 100 per cent (or almost) kn with a variation up to class V or VI, the dominigenes seem to be without effect. The same situation appears in the fourth group containing the vg^{no} compounds with vg and vg^{Nw} .

In attempting to find a connection between the different behavior of the higher and lower compounds with regard to the dominigene action and some other known factor, an important point concerning the development of the scalloped wings may be recalled. It was found that the maximum destruction of wing margin possible if the wing contour was still normal at the time of formation of the chitinous sheath soon after pupation, corresponds to classes V-VI. In the higher classes of scalloping the resorption began in the imaginal disk. The diagram shows that these same classes mark the limit of the action of the dominigenes. From this we might conclude that the dominigenes are concerned only with the processes which take place in the wing anlage after pupation.

It is a well-known fact that compounds of different multiple alleles usually show an intermediate behavior, whereas wild type is usually dominant. This fact has occasionally been mentioned as an argument against the senior author's quantitative conception of multiple allelomorphs. But other authors have maintained correctly that if there is a threshold beyond which only wild type can appear, the phenomenon fits into a quantitative conception of the alleles. The present experiments give interesting information on this point. If we measure dominance by the percentage of nonscalloped individuals, leaving out of account the amount of scalloping, we find the following relations:

Dominance of	Without dominigenes	With dominigenes
$ni/+$	100	97
$no/+$	100	75
$vg/+$	100	0
$Nw/+$	80	0

Dominance of wild type decreases in the series with the distance of the alleles, which becomes still more apparent if the dominigenes are present. From such a series we may conclude that the dominance of the wild type is a threshold affair, the threshold in the first series being reached if an allele as low as vg acts together with the wild allelomorph. Using a parallel with the phenomenon of sexuality we might call the $ni/+$ or $no/+$ phenotype a hyper-wild as compared with the $vg/+$. The presence of the dominigenes, however, shifts the threshold for wild beyond the level reached by the action of the $vg^{ni}/+$ compound.

A glimpse at figure 1 shows that the homozygous members of the series of alleles fit in perfectly with the heterozygous ones in regard to their phenotypic effect and its shift by the dominigenes. *This again shows that in this series dominance of the wild type and incomplete dominance are nothing but the*

consequence of a simple quantitative order with regard to the activity of the genes and their combinations during development, with a definite threshold for the production of the wild type.

In order to form as complete a picture of the phenomenon as present knowledge permits we must finally try to combine the interpretation of the embryological facts with the conclusions derived from genetic analysis. In a former paper the senior author tried to explain the development of the wing in the vg series in the following way: The normal growth and differentiation of the wing requires the presence of a substance, say a growth vitamin, which is produced in sufficient quantity during development and which enters into the wing bud at its base and spreads through the wing anlage in a stream, the bed of which is determined by the structural details of the organ. The vg alleles cause an insufficiency of this substance, which, with the further development of the organ, begins to be felt first at the tip. The parts where the insufficiency exists become degenerate and are resorbed. The earlier the production of the vitamin ceases or becomes inadequate, the greater the damage done. This damage follows, of course, the normal bed of the stream, thus giving the definite pattern of scalloping as found in the increasing series from nicked to no-wing. The series of phenotypes from no-wing to normal represents, as we expressed it, a moving picture of the path of the stream of the growth vitamin.

With this interpretation in view the entire body of genetical and embryological facts may be represented in the form of a diagram (fig. 2). On the abscissae the time and stages of differentiation are marked: second instar, third instar, pupation, determination, that is, the moment after which no change in pattern occurs. The top curve represents simultaneously the growth of the wing bud and the curve of production of the growth vitamin, which has to be present at each moment in a quantity sufficient to ensure normal development, that is, the production of the wild-type wing. For each of the three stages the minimum level is indicated for the quantity of the vitamin, which is always surpassed by the wild-type curve. The other curves represent the progress of different degrees of vitamin insufficiency. In the first group of three (ni-no), insufficiency and therewith resorption at the wing tip begin after pupation, when the production of vitamin has sunk below the minimum level; the abnormality spreads until the time of determination, when the normal parts of the wing differentiate, the degenerating ones having been resorbed. The result might be measured by the fraction of the minimum quantity which is present at this time, 10/11, 9/11, and so forth. These fractions of course measure the size of the remaining wing, and correspond, therefore, to the kn classes I-V and the phenotypes nicked to notched. The next two curves show the insufficiency beginning before pupation, so that at pupation a fraction, namely, 3/11 of the wing, is undergoing involution and resorption. At the time of determination resorption has gone up to 6/11 and 4/11, giving the respective kn classes. In the next curve insufficiency begins before the third, and the last two before the second, instar. If we regard the vg curve at the time of the second instar, we see that the wing bud is reduced to 10/11 of normal size, at the third instar to 8/11, after pupation to 5/11, and with further re-

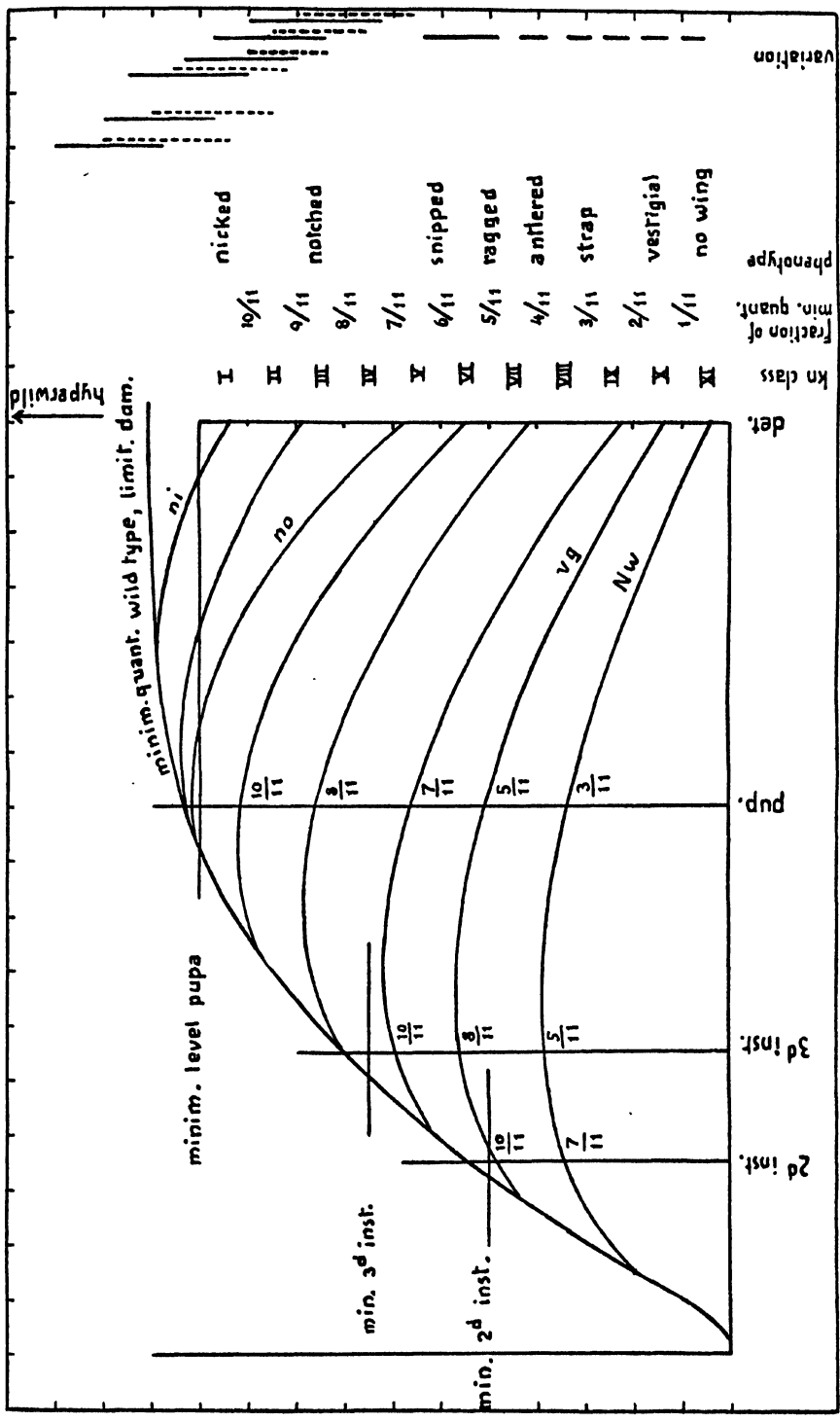


Fig. 2

sorption after pupation finally to 1/11–2/11. On the right side of this diagram the amount of variation for the different phenotypes is indicated as in figure 1; the broken lines indicate the effect of the dominigenes upon different genotypes. The diagram also demonstrates the dominance relations. If the curve for a heterozygote does not go below the level of the wild type, there is complete dominance of the wild type; if it sinks below, there are the different degrees of incomplete dominance. If the curve for the completely dominant heterozygote is intermediate between those of the parents, the curve for the wild race must be of the hyperwild type. As $Nw/+$ shows nicked individuals, the wild-type curve would have to be drawn ending at between 10 and 11 units above the end of the minimum curve.

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GENE AND CHARACTER
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BY
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GENE AND CHARACTER

VII. THE "NONHEREDITARY" *kn* EFFECT IN *DROSOPHILA*

BY

RICHARD GOLDSCHMIDT

IT IS A WELL-KNOWN FACT that in pure stocks of *Drosophila*, wild type or others, individuals occasionally appear with a nick in one or both wings, and that this, which we generally call the *kn* phenomenon, is not inherited. Our quantitative data on the occurrence of nick in different stocks have been reported in part II of this series of papers (1935). In working with the *kn* phenomenon, whether produced by modification (phenocopy, see part I, 1935), or as the effect of an allele of the *vg* series, or by the dominance-shifting action of the dominigenes for *vg* (see parts II, V, VI), one has to keep in mind that these "nonhereditary" *kn* individuals are present everywhere. It would therefore help the analysis of the entire phenomenon if the origin of these occasional *kn* individuals were known. A large amount of work has been done in this direction, which, though not leading to very startling results, had to be done for the sake of the completeness of the analysis. The first question to be decided is whether the phenomenon is really of a nonhereditary type. This means, first, that the known hereditary types of the same phenotype have to be tested; second, that other possibilities of a genotypic origin have to be looked for.

ARE *vg* ALLELOMORPHS INVOLVED IN THE PHENOMENON?

It is known that some of the lower *vg* allelomorphs have no visible phenotypic effect at all, even if homozygous, the effect becoming visible only in compounds, and that others produce only a small effect upon a small percentage of individuals. Such genes might then be present in a line, produce an occasional *kn* individual, and yet give no certain evidence of inherited effect if small numbers were bred. But a cross of such a *kn* individual with a higher member of the series, for example, vestigial, would reveal at once the presence of an allele of this series. Many such tests were made without success. Only in one combination, already reported in part II, was such an allele found—in a wild stock from Oregon.

ARE THE *vg* DOMINIGENES INVOLVED?

In parts II, V, and VI it was shown that the three dominigenes *ct^{do-vg}*, A, and B, which together shift dominance in the *vg* heterozygote toward intermediacy, that is, produce a *kn* effect, are genes which alone have hardly any effect. But if homozygous, at least for *ct^{do-vg}*, they have a small effect, making about 1 per cent of the individuals containing them nicked. As these dominigenes occur frequently in many stocks, they might occasionally become homozygous

and produce a few kn individuals. Therefore these individuals have to be tested for the dominigene, especially ct^{do-vg} . Three such males from spineless stock were crossed to females vg, homozygous for ct^{do-vg} , and heterozygous for B or A and B. If the ss ♂ kn contained ct^{do-vg} , about as many ♀ kn would be expected in F_1 as ♂ kn (for exact numbers see part VI). If the males did not contain the ct allele, only part of the sons could be kn, but no daughters except a few individuals (see part II). The results were:

Crosses		♀	♂	♀ kn	♂ kn
4587	♀ vg with dominigene × ♂ ss class I.....	43	25	26	47
4588	♀ vg with dominigene × ♂ ss class III.....	44	21	2	27
4578	♀ vg with dominigene × ♂ ss class I.....	65	50	—	22

One of the ♂ contained ct^{do-vg} , the others did not. (ct^{do-vg} occurs frequently in the ss stock and was originally isolated from it.) The effect is then not, or not typically, produced by the presence of the dominigene ct^{do-vg} . It is also not produced by A, B, because in this combination all F_1 ♂ would have been kn, as explained in detail in part V.

ARE OTHER KNOWN GENETIC AGENCIES INVOLVED?

It is known that flies carrying translocations frequently exhibit a kn effect, though in many individuals this is only one of the visible abnormalities. Therefore the possibility of translocations was kept in mind. The majority of kn individuals tested in different ways gave no results indicating a chromosome rearrangement. There was only one experiment in which after treatment with heat as many as 3 per cent kn individuals appeared. They turned out to be translocations.

A second test might have been similar. Females kn are much rarer than ♂♂, and often they are sterile. Once the rare occurrence of a kn ♀, class IV, was found in an ss stock and she turned out not to be entirely sterile. Crossed with a normal ♂ she produced 5 ♀, 3 normal ♂, 2 ♂ kn. All individuals were sterile. This, then, might also have been a translocation.

IS THE PHENOMENON OTHERWISE HEREDITARY?

The majority of the casual kn individuals, then, do not belong to the genetic types just discussed. The question is whether a genetic phenomenon is at all involved.

In part II it was demonstrated that at the time the earlier work was done the ss lines threw on an average 1 kn among 600 individuals; further, that the percentage was higher in mass cultures than in 1-pair cultures; and that some stocks usually produce higher percentages than others. These facts pointed to the possibility of a genetic basis.

The experiments to be reported here were begun after the following observation had been made. The lines which had thrown 1 kn among 600 flies for a long time were kept in mass culture. When a new control was made after many generations, it turned out that the kn individuals had become much

TABLE 1
SELECTION FOR PERCENTAGE OF kn

Genera- tions	A		B		C		D		E		F		G		H		I		K		L		M		N	
	no	kn	no	kn	no	kn	no	kn	no	kn	no	kn	no	kn	no	kn	no	kn	no	kn	no	kn	no	kn	no	kn
F ₁	273	-	115	-	300	-	250	-	190	-	280	-	235	-	180	-	230	-	250	-	170	-	240	-	200	-
F ₂	309	-	350	-	430	1	100	1	170	-	500	-	240	-	420	-	140	-	234	-	220	-	215	-	160	-
F ₃	280	-	150	-	270	-	155	-	210	-	510	-	190	-	360	-	255	-	430	1	120	-	220	-	220	-
F ₄	370	-	830	1	524	2	110	-	200	-	510	1	420	-	570	-	560	1	595	2	290	-	498	1	395	-
F ₅	160	-	530	-	237	-	105	-	295	-	190	-	400	-	410	1	390	-	470	-	140	-	260	-	89	-
F ₆	73	-	100	-	190	-	146	-	142	-	253	-	540	-	300	-	264	-	529	-	140	-	170	-	200	-
F ₇	321	-	-	-	123	-	188	-	-	-	253	-	450	-	303	1	402	-	620	-	-	-	150	-	289	-
F ₈	242	-	-	-	-	-	371	-	-	-	17	-	330	-	313	-	471	-	543	-	-	-	-	-	233	-
F ₉	-	-	-	-	-	-	181	-	-	-	239	1	150	-	189	-	138	-	345	-	-	-	-	-	203	-
F ₁₀	-	-	-	-	-	-	211	-	-	-	224	-	152	-	187	-	89	-	219	-	-	-	-	-	80	-
F ₁₁	-	-	-	-	-	-	77	-	-	-	59	-	-	-	86	-	159	-	238	-	-	-	-	-	84	-
Σa	2028	-	2075	1	2074	3	1894	1	1207	-	3035	2	3107	-	3318	2	3098	1	4473	3	1080	-	1753	1	2153	-

Σ 31,265 no, 14 kn=1 in 2,235.

rarer, though the environmental conditions had not been changed. Therefore a series of brother-sister lines was started with the intention of selecting lines with hereditary differences with regard to the percentage of kn. Table 1 gives the results. Thirteen lines were selected for 6-11 generations; 5 of these threw no kn individuals among a total of 9575 flies; the remaining 8 lines contained a few, altogether 14 among 21720, or 1 in 1500.

The first test was made with these lines by breeding from five pairs instead of from one. Thus, by crowding, the external conditions are changed to a certain degree, but not very much. Genetic conditions are also changed by allowing for more recombinations of eventual genes involved in the test as a consequence of promiscuity. Table 2 gives the results for six of the selected lines in five generations.

TABLE 2
FURTHER BREEDING FROM SELECTED LINES WITH AND WITHOUT CROWDING

Pair mating				Five-pair matings		
Line (table 1)	Generations	Individuals, total	1 kn among	Generations	Individuals total	1 kn among
A	8	2028	∞	5	5517	1872
C	7	2077	692	5	7783	355
F	11	3037	1519	5	6611	1101
G	10	3107	∞	5	7060	785
K	11	4476	1492	5	4720	∞
N	11	2153	∞	5	3999	1000

Table 2 shows: (1) In lines which had not thrown kn in many generations of pair culture, some kn individuals appear in the mass cultures. Once, also, the opposite occurred. (2) Line C, throwing the largest number of kn in pair matings, occupies the first place also in the mass cultures. This remained so in further experiments (see table 5). (3) On the average the mass cultures throw many more kn, as was also found previously. Whereas results (1) and (3) might be explained as the effect of a change of environment, or as the consequence of facilitating gene recombinations, the second result points strongly in the direction of a genetic basis for the phenomenon. In the next paper it will be demonstrated that in crosses involving lines with high and low percentages of kn the effect is recessive in F_1 and reappears in F_2 , thus revealing a genetic basis.

TEMPERATURE EXPERIMENTS

In a former paper (part I) it was demonstrated that the kn phenomenon can be produced at will in flies of the wild type by heat treatment of the pupa at a definite time. In connection with these experiments it was noted repeatedly that the offspring of such flies contained more kn individuals than was typical for the stock. There was, for example, a series of experiments made with the wild Oregon stock which at that time threw only 1 kn individual among 3000; in the offspring of heat-treated animals the kn individuals went up to 1 in 1000. A similar series within the spineless line produced six times as many kn

TABLE 3
RESULTS OF BREEDING FROM INDIVIDUALS TREATED WITH HEAT SHOCKS

Generation	Cross	Number of experiments with:		Total individuals				Number of bottles		Per cent kn bottles	Per cent kn	
		Pairs	Males	♀	♂	♀ kn	♂ kn	With kn	Without kn		All bottles	kn bottles
F ₁	kn × kn.....	40	—	3716	3471	2	4	5	35	2.5	0.08	0.6
	kn × kn.....	—	20	3217	3276	2	2	4	16	20.0	0.06	0.2
	kn × ss.....	10	—	980	843	1	—	1	9	10.0	0.06	0.6
	ss × kn.....	11	—	894	769	2	—	2	9	18.0	0.12	0.6
	kn × rucua.....	3	1	686	717	—	—	1	4	—	—	—
	kn × y er v f.....	3	—	331	284	—	—	1	3	—	—	—
	kn × no. brother.....	1	—	128	95	1	—	1	—	100.0	—	0.5
	no × no.....	7	—	753	797	—	2	2	5	29.0	0.13	0.4
	no × no.....	—	7	2313	2328	6	8	5	2	71.0	0.3	0.4
	(kn × kn) ²	149	—	16,644	13,528	38	24	43	106	29.0	0.21	0.7
F ₂	(kn × kn) ²	—	25	4784	4594	20	28	18	7	72.0	0.51	0.7
	(kn × ss) ²	23	—	3088	2798	5	7	9	14	39.0	0.20	0.5
	(ss × kn) ²	36	—	4563	4118	6	1	4	32	11.0	0.08	0.5
	(kn × ruc) ²	18	—	2231	2163	1	1	2	16	11.0	0.05	0.4
	(kn × y er v f) ²	15	—	2412	2010	1	2	3	12	20.0	0.07	0.4
	(no × no) ²	32	—	3883	3687	7	8	10	22	31.0	0.20	0.6
	(no × no) ²	—	8	1358	1203	8	5	4	4	50.0	0.50	1.0
	(kn × no) ²	19	—	2922	2515	1	1	1	18	5.0	0.04	0.8
	(ss × kn) ²	1	—	111	123	1	1	1	—	100.0	—	0.9
	Total.....			55,014	49,319	102	94					

individuals in the second generation after treatment. But in these experiments a number of sources of error were present. The experiments were therefore repeated with all precautions when the analysis of the kn phenomenon had proceeded to the point reported in my earlier publications.

The experiments were performed with ss stock which had been inbred for a long time and which in frequent controls was known to throw about 0.15 per cent kn individuals (0.12 per cent in 1-pair cultures, 0.18 in mass cultures). (For details on this line see Goldschmidt, 1935, part II.) With this line, kn individuals were produced as phenocopies by the action of heat (1935, part I), and these as well as their normal sisters and brothers were bred and outcrossed in different ways and in pair- as well as in mass culture, avoiding all possible sources of error.

The data which were obtained are summarized in table 3. The controls have already been mentioned. We find there data relating to F_1 and F_2 (one F_3) from treated parents, kn or normal, inbred or outcrossed in different combinations.

We note the following facts in F_1 :

(1) In pair cultures as well as in one series of mass cultures within the ss line, bred either from treated kn individuals or from crosses with untreated ones, three out of the four combinations threw only half as many kn individuals as the controls. The number of crosses excludes a chance result. (In a former series of crosses of the type $kn \times ss$ there was only 1 kn in 10,000 individuals.)

(2) In pair cultures the controls show that kn individuals appear in 10 per cent of the bottles; in the F_1 crosses, they appear in 14 per cent. Also, the mass cultures behave in this respect similarly to the controls, namely, 33 per cent contain kn.

(3) The percentage of kn individuals in bottles throwing kn is very constant (with one exception), namely, 0.4–0.6 per cent (1.1 per cent in the controls).

(4) In F_1 from outcrosses there appeared no kn individuals among 2000.

(5) The highest percentage of kn individuals and cultures was obtained from normal, but heat-treated, parents, namely, 0.13 per cent kn and 29 per cent kn bottles in pair culture, 0.3 per cent kn and 71 per cent bottles in mass culture, and 100 per cent bottles with one parent treated but normal.

In F_2 all combinations with the exception of the outcrosses (with rucua and y or v f) remain within the ss line. Whatever difference from the control is found must be the consequence of the heat treatment. As we have seen that the kn percentage is relatively constant in one brood or bottle, the decisive numbers are those relating to the number of broods with kn and the total average of kn depending upon it. These data for the ss line only are extracted in table 4 on page 321.

(1) As already mentioned, the number of bottles or broods containing kn is the same in F_1 as in the controls (10 per cent in pair cultures).

(2) In F_2 with pair cultures this percentage is sometimes tripled, and correspondingly the total average of kn individuals increased (F_2 kn broods 29, 39, 29 per cent; kn individuals, 0.21, 0.20, 0.20 per cent). Mass cultures show a

similar effect, for example, for $(kn \times kn)^2$ 72 per cent bottles and 0.51 per cent average.

(3) There is one exception: if the mother of F_1 was an untreated $ss \text{ } \varnothing$, F_2 was like F_1 and the controls.

(4) We have already seen in F_1 a specially high percentage of kn if the parents were treated, but normal, individuals. In F_2 the results are similar to the others.

These data allow a number of conclusions: Neither in F_1 nor F_2 has the number of kn individuals within a bottle been increased; this shows that

TABLE 4
SUMMARY OF DATA CONTAINED IN TABLE 3

Combination	Per cent kn bottles	Per cent kn indi- viduals	Number	Combination	Per cent kn bottles	Per cent kn indi- viduals	Number
Control pairs	10.2	0.12	11,029	$F_2 (kn \times ss)^2$ pairs	39.0	0.20	5,898
Control mass	60.0	0.18	15,649	$F_1 ss \times kn$ pairs	18.0	0.12	1,665
$F_1 kn \times kn$ pairs	12.5	0.08	7,193	$F_2 (ss \times kn)^2$ pairs	11.0	0.08	8,688
$F_1 kn \times kn$ mass	20.0	0.06	6,497	$F_1 no \times no$ pairs	29.0	0.13	1,552
$F_2 (kn \times kn)^2$ pairs	29.0	0.21	30,226	$F_1 no \times no$ mass	71.0	0.30	4,655
$F_2 (kn \times kn)^2$ mass	72.0	0.51	9,426	$F_2 (no \times no)^2$ pairs	31.0	0.20	7,585
$F_1 kn \times ss$ pairs	10.0	0.06	1,824	$F_2 (no \times no)^2$ mass	50.0	0.50	2,574

whatever genetic process brings about this small kn effect, it has not been influenced by temperature. But, there is the increase in the number of broods which show the effect, and this increase is found only if the line comes from a treated female. This leads to the conclusion that the heat treatment resulted in a plasmatic change of the type of dauermodification which enhances the action of the genes in question. If, for example, the recombination of four recessive genes, otherwise without effect, would be needed to produce the kn effect, the changed protoplasm would permit an effect with three genes. Something of this general type is bound to give the explanation, which, however, is difficult to prove without a successful experiment in selection. As noted earlier, the outcrossing with the rucuca and y cr v f lines shows that the genes involved are recessive and recombine in F_2 (see table 3).

TEMPERATURE EXPERIMENTS WITH SELECTED STOCK

We have already mentioned that in mass cultures which had always thrown a constant percentage of kn , the typical percentage of kn individuals fell after a long interval without control. The behavior of selected lines from this stock has been reported. Besides, 202 1-pair cultures taken from this stock were bred. Seventeen of these, or 8.5 per cent, contained kn individuals, nearly the same number as in the original stock. But the average number of kn individuals was 0.04, or about one-third of the number typical for the stock originally. It has to be assumed that the stock had become more heterozygous for the different recessive genes involved in the phenomenon; the successful selection of lines without kn which threw kn again when bred in 5-pair cultures favors such a view.

TABLE 5—(Concluded)

Line	Exp.	F ₁										F ₂ from F ₁									
		kn×kn					no×no					kn×kn					no×no				
		+	kn	+	kn	+	kn	+	kn	+	kn	+	kn	+	kn	+	+	kn	+	kn	+
C	1	406	1	1420	6	947	1	220	—	390	2	579	1	1235	2	1471	3	424	1	226	3
	2	510	2	733	1	625	1	222	3	439	6	546	1	409	—	646	—	2002	6	241	—
	3	307	1	746	3	379	—	624	—	430	—	302	—	266	2	313	1	715	—	334	1
	4	1183	7	509	3	701	—	379	—	445	1	1338	2	826	4	735	1	285	1	336	2
	Sa	2906	11	3408	13	2652	2	3446	3	1704	9	2765	4	2736	8	3165	5	3426	8	1137	6
F	1	544	—	953	—	580	1	633	—	359	—	697	—	673	1	701	—	678	3	227	—
	2	621	—	842	1	726	—	854	—	463	1	667	—	1053	3	656	—	885	—	237	1
	3	286	1	87	—	206	2	979	—	509	—	414	—	340	—	267	—	1138	3	316	—
	4	1543	—	1237	1	1419	1	552	—	322	—	1410	1	1077	—	1580	1	667	—	365	2
	Sa	2994	1	3119	2	2931	4	3018	—	1653	1	3188	1	3143	4	3204	1	3368	6	1145	3
N	1	724	1	861	6	1114	3	1028	3	379	—	857	5	1273	5	996	1	687	1	337	—
	2	1383	3	873	—	1606	1	2904	3	477	1	1507	3	703	1	1488	1	2339	6	367	1
	3	885	3	1525	3	795	4	344	—	394	—	512	2	850	3	697	1	394	—	219	1
	Sa	2992	7	3259	9	3515	8	4276	6	1230	1	2876	10	2826	9	3181	3	3420	7	923	2

TABLE 6

SIMPLIFIED DATA COMPILED FROM TABLE 5

Line	Both parents treated				One parent treated				Controls				1 kn : x individuals in						
	F ₁ +		F ₂ +	F ₂ kn	F ₁ +		F ₂ kn	F ₁ +	F ₂ +	F ₂ kn	F ₁ kn	F ₂ +	F ₁ kn	Both parents treated		One parent treated		Controls	
	F ₁ +	F ₁ kn	F ₂ +	F ₂ kn	F ₁ +	F ₁ kn	F ₂ +	F ₂ kn	F ₁ +	F ₂ +	F ₁ kn	F ₂ +	F ₁ kn	F ₁	F ₂	F ₁	F ₂	F ₁	F ₂
I (G).....	7652	6	6031	5	7095	5	6560	5	1843	4	1307	2	1275	1206	1420	1313	461	654	
II (K).....	5641	2	5617	2	6280	9	7031	8	1250	—	1202	—	2820	2808	699	875	∞	∞	
III (A).....	5854	3	5892	3	5785	7	6349	4	1740	2	1254	—	1951	1964	827	1588	870	∞	
IV (C).....	6314	24	5501	12	6089	5	6591	13	1704	9	1137	6	263	458	1221	507	189	189	
V (F).....	6113	3	6331	5	5949	4	6572	7	1653	1	1145	3	2038	1266	1488	940	1653	382	
VI (N).....	6251	16	5702	19	7791	14	6601	10	1230	1	923	2	391	300	558	661	1230	462	

The temperature experiments were repeated with six of these selected lines (see table 1), namely, A, G, N, which did not throw kn in pair culture, and C, F, K, which did. The experiments were all performed with 5-pair cultures to enhance the chances of recombination of the genes in question and thus to equalize the genetic background of the different experiments. Of course, the controls were treated in the same way. In each one of the six lines, four parallel experiments were run, marked 1-4 in the tables. Each of these twenty-four cultures was exposed to high temperature, exactly as described in part I of this series. From the treated animals, four combinations were always bred, namely, both sexes phenotypically kn, both wild type, kn ♀ × normal ♂ from untreated controls ss, and reciprocal. The data are found in table 5, and from this table 6 has been compiled, in which the data have been simplified. Only F_1 and F_2 have been recorded in the tables because the generations up to F_6 , when the experiment was finished, do not show anything of interest. Tables 5 and 6 convey the following information:

(1) Comparing the column "Both parents treated" with the controls (table 6), we see a general parallelism in the results. If there was a comparatively large number of kn in the controls, the same was true for the experiments (line IV); no kn in the controls (line II) meant also very few in the experiments; and intermediate numbers in the controls were paralleled in the experiments (lines I, III, V).

(2) With the exception of line VI, the absolute number of kn was much below the numbers for the controls.

(3) When only one of the parents was treated, the result was either the same as just mentioned (lines I, VI), or the opposite (line II), or rather irregular.

(4) In one experiment (table 5, line A, exp. 2, F_2) an unusual number of kn individuals appeared (1 in 35). The offspring proved the presence of a translocation.

(5) The maternal influence observed in the former experiments was not visible.

The results of this series of experiments do not confirm the former results. Neither was the kn percentage increased by the temperature shock; nor was there a difference in reciprocal crosses nor a different behavior in F_1 and F_2 . They show, however, that the phenomenon has some genetic basis, as the experimental lines behaved like the controls which had been selected for eleven generations. It is not known why the two rather large series of experiments yielded different results. Of course, the stock had changed genetically, as reported, but this does not lead to a plausible interpretation of the difference. The most probable explanation is that environmental factors play a rather large rôle in producing the kn effect, and their action is different if a different genetic basis is given. It seems hardly worth while to continue this investigation of the so-called nonheritable nicks.

SUMMARY

(1) In the ss line, and much less frequently in other lines of *Drosophila*, individuals with a nick at the tip of the wings appear regularly. The phenomenon was supposed not to be inherited.

(2) One line for a long time threw a constant percentage of these individuals. After a number of uncontrolled generations this percentage had dropped to one-third the original number and remained constant.

(3) From the latter stock, lines with and without kn individuals could be isolated by selection.

(4) Lines which did not produce kn individuals for eleven generations of pair matings produced some if five pairs were bred together.

(5) All data point to the assumption that the phenomenon has a genetic basis, namely, a number of recessive genes recombining for the visible effect.

(6) A successful selection was nevertheless impossible. As the kn females were frequently sterile with kn males, a large accumulation of these genes seems to be impossible.

(7) Outcrossing experiments show the effect to be recessive.

(8) The assumption can be excluded that vg alleles or the dominigenes for vg are involved. But once in wild stock [Oregon], a kn individual turned out to contain a vg allele; in one other, a high percentage of kn after heat treatment turned out to be the effect of a translocation.

(9) The offspring of heat-treated animals, showing or not showing the kn effect, contained in one experimental series an increased number of kn; in another series with a different genetic background there was no increase. Besides having genetical differences, the phenomenon is probably highly modifiable. The different results obtained, even under conditions constant to all intents and purposes, may therefore be without meaning.

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GENE AND CHARACTER
VIII. A SELECTION EXPERIMENT WITH
DOMINIGENES

BY
RICHARD GOLDSCHMIDT

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GENE AND CHARACTER

VIII. A SELECTION EXPERIMENT WITH DOMINIGENES

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DOMINIGENES are modifying genes which shift the phenotype of a heterozygote in favor of dominance or increased dominance of one of the alleles. In the special case which we have been studying for some time, the dominigenes, exerting influence upon the vestigial series (Goldschmidt, 1935, 1937), act in favor of the recessive type, shifting the heterozygote away from wild type. Three such dominigenes are involved, one a cut allele, ct^{do-vg} , in the first chromosome, and two autosomal genes A and B in the second and third chromosomes. The shifting effect takes place if ct^{do-vg} is present (in the female homozygous), and besides, at least one dose of A and B. More A and B up to homozygosity increases the effect, which in addition shows a different threshold in females and males. In Fisher's theory of the evolution of dominance it is assumed that dominigenes tending to enhance the dominance of the wild type have a selective value in the heterozygote. Here we are dealing with dominigenes which act in the opposite way from wild type. It would then be expected that they have a negative selection value in the heterozygote. In his criticism of Fisher's work, Wright (1934) discusses the questions which arise if the modifiers have any effects on their own account which are subject to selection. He concludes, as does also Haldane, that the pressure due to such selection is certain to take precedence over that due to its effect on the rare heterozygote. It is therefore of interest to study the effects of selection upon such modifiers, the dominigenes, which can be done with the vg dominigenes.

Two facts were ascertained with respect to this question in my previous work. First, there is a noticeable sublethal effect upon the homozygous classes if all dominigenes are present in homozygous condition, that is, on the classes $+/+$ and vg/vg in a segregating F_2 , which ought to contain 1 wild : 2 intermediate : 1 vg . But the detrimental effect is not found in the heterozygote $vg/+$ plus dominigenes, which is intermediate. This does not suggest selective value in favor of wild type. Second, the different dominigenes are widely scattered in the different lines which were tested (see Goldschmidt, 1935, part II) and in some lines they were frequently present without phenotypic effect. Their continuous presence in unselected stock does not point to an appreciable negative selective value. It was decided, therefore, to run a simple selection experiment which might give further information.

The populations which were to be tested were made up to contain at the start a different distribution of dominigenes. They were started by crossing an inbred wild-type line (Oregon) with a line homozygous for the dominigenes and otherwise wild type (Doho). In order to have a different admixture of

dominigenes to the population, the lines were started with different numbers of Oregon and Doho (= homozygous dominigene line) : (1) 10 couples Oregon, 10 couples Doho; (2) 8 Oregon, 10 Doho; (3) 6 Oregon, 10 Doho; (4) 4 Oregon, 10 Doho; (5) 2 Oregon, 10 Doho; (6) 1 Oregon, 10 Doho. All flies were of the same age (15 days), came from pair matings, and had been bred identically; the females were virgins. They were left together in one vial three days for promiscuous mating and then transferred to bottles, from which they were removed after five days; the bottles were kept at 25°, all other conditions being as constant as possible. After fifteen days the next generation was started.

To test different degrees of selection-pressure upon these different populations, different amounts of crowding were provided in the following generations by breeding from 5, 10, 20, 50 couples in further generations, everything else being equal. On the fifteenth day 5 or 10, and so forth, couples were taken at random from the bottles and started as a new generation in the same way as described before. In the following generations, of course, the same amount of crowding was continued in each line, that is, always 5 or 10, and so forth, couples in each generation. Thus, 24 lines of population were run with 6 different mixtures at the start and 4 different types of crowding in each. These lines were run exactly alike for 28 generations and then tested for dominigenes. For this purpose 20 virgin females from each of the 24 lines were crossed to males homozygous for all dominigenes but heterozygous $vg/+$. One-half of the offspring would be $vg/+$ again and would show the presence of the dominigenes. As all received A and B once from the father, they would show the effect (scalloped wings) if they contained ct^{do-vg} . If the mother was homozygous for this gene, all sons and daughters $vg/+$ would be scalloped; if the mother was heterozygous, half of them would be scalloped (i.e., $\frac{1}{2}$ or $\frac{1}{4}$ of all offspring). If the mother did not have ct^{do-vg} , only wild type would appear. There is, however, some deviation from these expectations because some males with A/A b/b are kn. If, however, exactly one-half of the females and males turned out to be kn, it must be assumed that either A or B are homozygous and that the mother contained one of these genes. Besides, the presence of A and B in the mother will be tested by the grade of the scalloped offspring. The presence of class I only with a slight variation to I and III means that the mother contained no A or B (offspring all $A/+$ $B/+$); higher classes mean that the mother also introduced A or B in heterozygous or homozygous condition. The details of this expectation may be easily worked out.

The first question to be asked is concerning the viability of the different combinations. This should give certain information as well about the ability of the genetically different populations to hold their own, with respect to the deleterious effect of crowding. Table 1 contains the number of individuals in each bottle counted at identical times under constant conditions in the 27 generations of the experiment and for the 24 types of population. 10/2 means we start with 10 pairs Doho and 2 pairs Oregon, and the other combinations are read accordingly. The numbers of pairs per bottle measure the crowding. The

original cross, the parents of the first generation of this experiment, gave under the same conditions :

<i>Combination</i>	<i>10/1</i>	<i>10/2</i>	<i>10/4</i>	<i>10/6</i>	<i>10/8</i>	<i>10/10</i>
Offspring	206	284	245	277	238	181

To save space, table 1 does not give the numbers for each culture in 27 generations but only the totals and the average per generation, there being no particular differences visible between the generations.

TABLE 1
TWENTY-SEVEN GENERATIONS OF MASS CULTURE WITH DOMINIGENES

Combination	Number of couples	Number in 27 generations	Average per generation	Group average
10/1.....	5	8090	300	1817
10/2.....	5	7988	296	
10/4.....	5	8419	312	
10/6.....	5	8284	307	
10/8.....	5	7935	294	
10/10.....	5	8342	309	
10/1.....	10	7506	279	1505
10/2.....	10	6205	230	
10/4.....	10	6753	250	
10/6.....	10	6647	246	
10/8.....	10	7073	299	
10/10.....	10	6460	239	
10/1.....	20	5209	156	1236
10/2.....	20	5546	205	
10/4.....	20	5676	210	
10/6.....	20	5420	201	
10/8.....	20	5678	210	
10/10.....	20	5829	216	
10/1.....	50	5691	211	1228
10/2.....	50	5290	159	
10/4.....	50	5481	203	
10/6.....	50	5561	206	
10/8.....	50	5394	200	
10/10.....	50	5726	212	
Total.....		156,203		

Table 1 shows: (1) There is no significant difference in viability within one selection group. The genetic composition of the six types of population is either not very different or the difference is without influence. (2) There is a typical and constant inverse relation between crowding and viability, the greatest number of individuals being bred with least crowding, and the smallest number with most crowding. However, there is no further difference found if the crowding is increased from 20 to 50 couples.

Table 2 tabulates the results of testing the constitution of 24×20 females after 27 generations by the method outlined above. The first two columns indicate again the same twenty-four experimental types as in table 1. The following three columns indicate how many of the twenty females tested in each group contained the dominigene ct^{do-vs} in homozygous or heterozygous

TABLE 2
TESTING FEMALES FOR DOMINIGENES AFTER TWENTY-SEVEN GENERATIONS

Oregon type in first cross	Couples	Number of tested ♀♀ out of 20, first chromosome			dto kn classes		A or B in mother	Total number			
		$\frac{ct^{do-vs}}{ct^{do-vs}}$	$\frac{ct^{do-vs}}{+}$	$\frac{+}{+}$	I-III	I-V		♀	♂	♀ kn	♂ kn
1	5	17	2	1	1	2	2	978	931	281	466
2	5	14	5	1	5	—	—	976	986	331	527
4	5	9	10	1	5	1	—	927	989	251	443
6	5	16	4	—	6	1	1	892	904	272	450
8	5	8	10	2	6	1	—	896	865	253	382
10	5	2	6	12	3	—	—	854	912	134	187
1	10	17	2	1	2	4	1	824	768	197	410
2	10	16	2	2	5	1	2	845	844	247	427
4	10	15	3	2	11	4	3	693	769	246	379
6	10	6	7	5	8	—	—	999	893	197	310
8	10	7	8	5	8	3	—	832	1065	360	517
10	10	3	11	4	12	2	2	469	444	133	172
1	20	12	4	4	8	1	—	618	626	148	246
2	20	9	6	5	10	3	4	456	434	111	208
4	20	10	9	1	4	1	—	547	516	124	227
6	20	16	3	—	7	—	2	708	678	205	353
8	20	10	6	4	10	3	1	582	610	158	255
10	20	14	4	1	10	2	—	702	719	220	387
1	50	7	9	3	10	—	1	607	601	167	220
2	50	17	3	—	13	2	2	662	657	277	426
4	50	11	6	2	7	—	2	425	484	90	217
6	50	13	5	1	10	2	—	295	354	89	189
8	50	15	4	1	9	2	—	786	750	203	375
10	50	6	5	9	2	—	1	489	500	70	158
Total								17,064	17,209	4,764	7,931

condition, as judged by the number of kn individuals in the offspring (nearly $\frac{1}{2}$ = homozygous, nearly $\frac{1}{4}$ = heterozygous, none or a few single ones = no dominigene). The next two columns indicate how often the higher kn classes were represented; in this test, unfortunately, only part of the cultures were recorded. The last column indicates how often exactly $\frac{1}{2}$ kn were found, proving that A or B was present in the mother.

The results in this table are striking. There was no bottle in which ct^{do-vs} was eliminated. Among twenty individuals this dominigene was always rep-

resented in homozygous or heterozygous condition. Relatively few individuals (about $\frac{1}{4}$) proved to be without this dominigene. In the first two series (with 5 and 10 couples as parents) there is a certain regularity visible; with increasing numbers of wild-type parents in the original cross, the number of homozygotes for the dominigene decreases. This would be expected if dominigenes and their wild allelomorphs had no different selection value. But in the last two groups of mass culture this rule does not hold. This might be interpreted even as indicating positive selective value of this dominigene under conditions of crowding. There is certainly within the limits of the experiment, which is in no way exhaustive yet, no trace of a negative selective value of the dominigene ct^{40-78} detectable.

It is more difficult to draw reliable conclusions in regard to A and B. As the last column indicates, some females scattered over about half of the twenty-four populations and over all groups still containing A or B. There certainly was no female found with two or more of these genes, because in this group the kn offspring would have been mostly of the higher classes. But wherever class IV or V appeared it was in small numbers. Thus it is possible, though not yet proved, that A and B actually have a negative selective value. This conclusion is not improbable, because of the sublethality of the complete homozygotes, proof for which already has been established.

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EXPLANATION OF PLATES

PLATE 15

Stages in the development of the scalloped wing, class V, *Drosophila melanogaster*.

PLATE 16

Stages in the development of the scalloped wing, class VI, *Drosophila melanogaster*.



PLATE 17

Stages in the development of the scalloped wing, classes VII-VIII, *Drosophila melanogaster*.

PLATE 18

Stages in the development of the dumpy wing, *Drosophila melanogaster*.

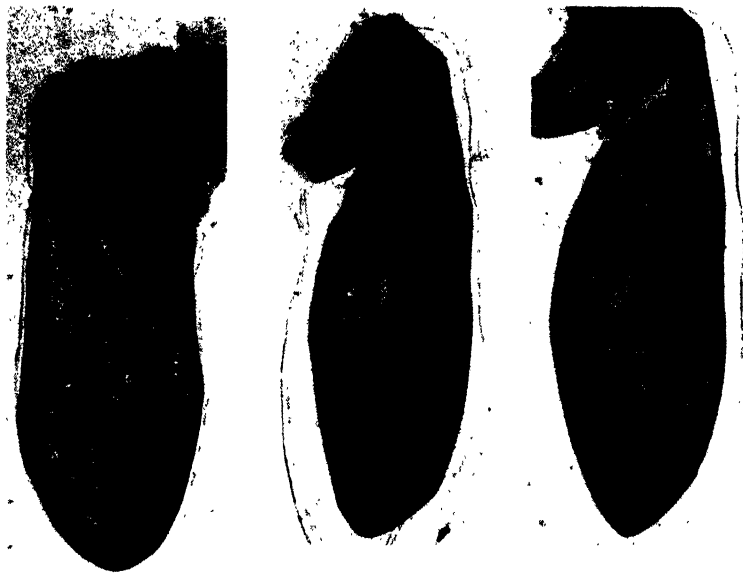


PLATE 19

Stages in the development of the wing with pointed tip, *Drosophila melanogaster*.

PLATE 20

Stages in the development of the expanded wing, *Drosophila melanogaster*.



**THE MORPHOLOGY, DIVISION, AND
CONJUGATION OF THE SALT-MARSH
CILIAE FABREA SALINA HENNEGUY**

BY

JOHN MARSHALL ELLIS

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CONTENTS

	PAGE
Introduction.....	343
Acknowledgments.....	343
Review of literature.....	343
Technique.....	344
Observations on the living animal.....	345
Morphology.....	350
Division.....	356
Conjugation.....	359
Summary.....	371
Literature cited.....	373
Explanation of plates.....	377

THE MORPHOLOGY, DIVISION, AND CONJUGATION OF THE SALT MARSH CILIATE *FABREA* SALINA HENNEGUY

BY

JOHN MARSHALL ELLIS

INTRODUCTION

IN THE SUMMER OF 1931, in a study of the Protozoa in some salt-marsh water from El Cerrito inlet, on the edge of San Francisco Bay, the attention of the writer was attracted to a number of large greenish black heterotrich ciliates. Investigation showed that the ciliate was *Fabrea salina*, and a search through literature brought to light the original papers describing the animal.

A few days after the material was brought into the laboratory an epidemic of conjugation was observed. Cultures were made immediately and were kept going through the summer. In the summer of 1932 the work was resumed at the University of California, and continued for two years.

ACKNOWLEDGMENTS

I wish to express my appreciation to Professor Harold Kirby, Jr., under whose direction this work was carried out, for his constant interest and helpful advice. To Professor C. A. Kofoid I am indebted for the use of his private library and for much helpful criticism. I should also like to express my thanks to Professor S. C. Brooks and to Dr. Mildred Bush for valuable suggestions throughout the course of this work, to Mrs. E. A. Scott for the use of special optical equipment, and to Miss Dorothy G. Harris for assistance with some drawings.

REVIEW OF LITERATURE

Fabrea salina, a heterotrich ciliate found only in concentrated salt water, was first described by Stepanow (1885) as a variety of *Climacostomum* St. The following year Stepanow (1886) again referred to the ciliate as *Climacostomum* n. sp., in a description of the fauna of the salt lakes of Slaviansk.

In 1890 Henneguy gave the first complete description of the ciliate, which he found in the salt marshes of Croisie, and named *Fabrea salina* in honor of M. Fabre-Domergue. A brief description was given in a communication to the Paris Société de Biologie, séance du 15 novembre, 1890 (Henneguy, 1890a). A complete description, with 13 plate figures, appeared in the December 2, 1890, issue of the *Annales de Micrographie* (Henneguy, 1890b). Later Balbiani (1893), in a paper on regeneration in ciliates, described several experiments on *Fabrea*.

Geza Entz (1904), in a paper on the fauna of the salt seas near Torda, described *Fabrea* under the name *Climacostomum stepanowii*. From drawings sent to him by Stepanow, he was certain, he stated, that his species and the one described by Stepanow were the same.

Bujor (1904) included *Fabrea* in the list of Protozoa found in the salt lakes of Rumania.

A further study of *Fabrea* was made by Fauré-Fremiet and his students (Fauré-Fremiet, 1911 and 1912; Donnasson and Fauré-Fremiet, 1911).

In 1929, Kalmus described a salt marsh ciliate under the name *Bursalinus synspiralis*. In a later article Kalmus (1932) stated that the paper of Entz had come to his attention, and that the form described by him was undoubtedly the one described by Entz.

Kahl (1932) stated that *Fabrea* was reported by Dr. J. E. Lynch as having been found near New Orleans, but a letter from Dr. Lynch informs me that this is an error, and that his report to Kahl was of his finding *Fabrea* in the marshes of San Francisco Bay. *Fabrea* was included in the recent survey of salt marsh Protozoa by Kirby (1934) from the same region, and from Elkhorn Slough near Monterey.

TECHNIQUE

Culture methods.—*Fabrea* can be grown quite easily in cultures at laboratory temperature. The best medium is made with 250 cc. sea water concentrated to a density of 1.050, to which are added 30–40 grains of wheat. The cultures are kept in covered glass refrigerator dishes or in ordinary finger bowls.

It has been possible to induce conjugation epidemics in most cultures. The most commonly used method of dilution with distilled water is rarely successful. But when a rich culture in a medium of 1.070–1.090 is centrifuged and transferred to a fresh medium of 1.050, a conjugation epidemic will usually occur within two or three days. Succeeding epidemics may be induced in the same culture at intervals of about eight or ten days. Since nuclear reorganization after conjugation requires about a week, it seems that the animals are capable of conjugation immediately after reorganization. There is no high death rate of exconjugants, as has been described in many ciliates.

Vital staining.—*Fabrea* has been stained so that the effects of both vital stains and poisons may be observed. For the purposes of vital staining, neutral red, Bismarck brown, Janus green B, methyl violet, and brilliant cresyl blue have been used.

Permanent preparations.—For temporary preparations aceto-carmin was used in the study of the progress of reorganization after conjugation. Sudan III and Scharlach R were used in testing for fats, and chlor-zinc-iodide for glycogen.

Of fixatives used, all were valuable for various details, but Schaudinn's, Bouin's, and Flemming's fluids gave the best general results. For nuclear details, iron haematoxylin and Feulgen stains were most satisfactory. The

Borrel stain was good, but the red and green were variable in their selectivity for nuclear components, even in ciliates stained by the same time schedule. The Champy-Kull method gave the best results for mitochondria, and the Mann-Kopsch and Kolatchev methods for osmiophilic inclusions.

For a study of the pellicular pattern, preparations were made according to the methods of Bresslau (1921), Klein (1926), Lund's (1933) modification of Gelei and Horvath (1931), Chatton and Lwoff (1930), and Kidder (1933a). For a study of internal structure and neuromotor fibrils, iron haematoxylin and Mallory's triple stain gave best results. The Yabroff (1928) technique gave better results than did other silver methods, but did not show any external pellicular pattern comparable to a "silverline" system.

OBSERVATIONS ON THE LIVING ANIMAL

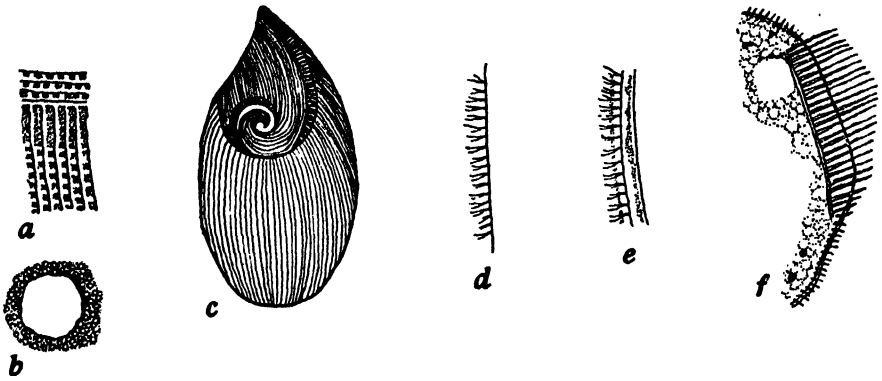
External characters.—*Fabrea salina* varies in size from dwarf forms of $50 \times 75\mu$ to giants of $320 \times 400\mu$, averaging about $120 \times 180\mu$. Conjugants and ex-conjugants average about $90 \times 150\mu$. None of the forms studied reached the size of $300 \times 600\mu$ reported by Entz (1901).

Fabrea is pyriform, with the anterior part excavated by a spirally wound adoral zone which sinks deeply into the body on the right side. The numerous, closely set membranelles are each about 10μ in width. The cilia making up the outer part of a membranelle are about 15μ , and the inner ones 10μ in length.

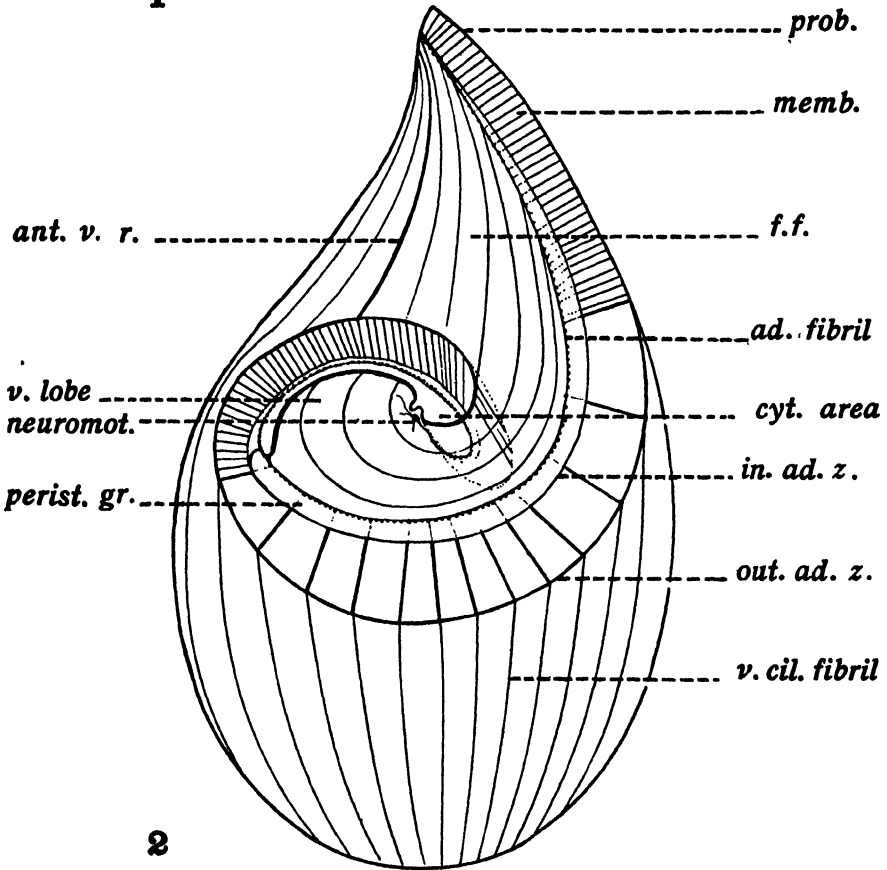
To the right of the adoral zone lies the frontal field. There is a ridge on the ventral surface from the anterior tip of the animal along the mid-line of the body to the anterior border of the coiling adoral zone (text fig. A, 2). On the right of this ridge the ciliary rows run toward the posterior end of the animal. It has not been possible to find a ridge running from the right border of the adoral zone horizontally, as described by Henneguy (1890b), and shown in text figure A, 1c, which is drawn from his plate. The ciliary rows of the left slope of the ventral ridge follow the direction of the coiling adoral zone (pl. 15, fig. 1). When the adoral zone leaves the frontal field and sinks into the buccal pouch, the frontal field is continued as a lobe which forms the posterior wall of the buccal pouch. This will be referred to as the ventral lobe.

A clear stripe separates the posterior ciliary row of the frontal field from the anterior border of the adoral zone. This appears to be homologous with a similar band in *Stentor coeruleus*, described by Schuberg (1890) and Johnson (1893) as the true peristomial area. This will be called the peristomial band. It is free from cilia, but is bordered on the side next to the frontal field by a closely set row of comparatively long cilia. A similar band was described by Maier (1903) in *Stentor coeruleus*, the "Randstreife," bordered by a "Randcilienreihe."

The rows of cilia on the ventral lobe continue over its anterior edge into the buccal pouch. The cilia become longer at the base of this lobe, and some of them extend into the endoplasm as "pharyngeal fibrils." The innermost cilia of the "Randcilienreihe," the ciliary row which runs parallel to the adoral zone, also extend into the endoplasm, forming with those described above an



1



2

Fig. A, 1 and 2. *Fabrea salina*.

(Explanation of Fig. A on page 347.)

incomplete circle of fibrils which guide the food deep into the endoplasm. The floor of the buccal pouch is not ciliated, but seems to be a naked zone of protoplasm through which food is ingested. There is no preformed mouth opening.

The pharyngeal fibrils have been described by Fauré-Fremiet (1912). The observations of the writer are in accord with those of Fauré-Fremiet, except that the "faisceau annulaire" is found to be an incomplete circle formed in part by the "Randeilien" and in part by the cilia coming off from the base of the ventral lobe. These cilia spread out in a crescent-like fashion (pl. 15, figs. 4-7), forming a circular fibrillar ring. Sections through this region would therefore show two bundles of fibrils, as indicated by Fauré-Fremiet (1912, Fig. VI.). The fibrils are easily distinguished in the living animal, and it can be seen that they diverge to fit the size of the bolus of food that is being swallowed.

The surface of the body is covered with a very dense coat of fine cilia. The ciliary rows are continuous on the dorsal surface, running in almost parallel rows from the anterior to the posterior ends of the body. Anteriorly they join obliquely the left border of the curving adoral zone. The ciliary rows of the ventral surface fall into two groups. Those of the frontal field end anteriorly at an oblique angle with the adoral zone, and posteriorly may end on the edge of the curving adoral zone or circle around the left lobe of the buccal pouch to extend over the ventral lobe on the posterior wall of the pouch. The ciliary rows of the posterior ventral surface run from the outer border of the horizontal curve of the adoral zone to converge with the lateral and dorsal rows at the posterior end of the body. Not all of the ciliary rows do reach the posterior end, some of them converging obliquely with other rows.

The most striking aspect of the external surface is the appearance of the longitudinal striations of the ectoplasm. This is caused by the alternation of dark and light bands under the pellicle, the "Rippenstreifen" and "Zwischenstreifen" of Bütschli (1889). In the dark bands the ectoplasm is filled with very small granules. According to Henneguy (1890b), these are quadrangular areas separated by narrow light spaces (text fig. A, 1a). My own observations, on both living and stained specimens, show that the dark granules are continuous on the left side of the band, the quadrangular areas separated by light spaces making up the right portion of the band (pl. 17, fig. 30). It is from

Fig. A, 1 and 2. *Fabrea salina*.

1. Diagrams of *Fabrea salina* (from Henneguy, 1890b).

a. Part of surface showing granular bands in the region of the adoral zone.

b. Optical section, showing food vacuole surrounded by endoplasm.

c. Ventral surface, showing longitudinal striations of the body and the peristomial field.

d. An isolated fibril to which are attached pairs of cilia.

e. Sketch showing relationship of fibrils to granular bands.

f. Section through the adoral zone of membranelles.

2. The fibrillar system of *Fabrea salina*. Abbreviations: *ad. fibril*, adoral fibril; *ant. v. r.*, anterior ventral ridge; *cyt. area*, mouth area of buccal pouch; *f. f.*, frontal field; *in. ad. s.*, inner border adoral zone; *memb.*, membranelles; *neuromot.*, neuromotorium; *out. ad. s.*, outer border adoral zone; *perist. gr.*, peristomial groove; *prob.*, proboscis; *v. cil. fibril*, ventral ciliary fibril; *v. lobe*, ventral lobe frontal field.

the light spaces between the quadrangular areas, rather than from the clear stripes ("Zwischenstreifen"), that the cilia seem to arise. The body cilia are of approximately the same length over the entire surface of the body, averaging 12μ . They are longer on the border of the peristomial band, and on the wall of the ventral lobe where the ciliary rows turn in to the buccal funnel.

As observed by Henneguy (1890b), the cilia come off in pairs, but they can be traced to their disappearance in the pellicle as separate threads which seem to be anchored in separate basal granules. It has not been possible to find sections showing two cilia arising from a single granule. There is, as Henneguy describes, a longitudinal fibril connecting the basal granules (text fig. A, 1d). These fibrils are often seen in crushed animals in osmic preparations. They are called myonemes by Henneguy (1890b), but are considered by the writer to be interconnecting fibrils of the neuromotor system.

As Henneguy (1890b) observed, the clear bands of the longitudinal stripes are continued across the adoral zone as the bases of the membranelles (pl. 15, fig. 1). The "Rippenstreifen" are also continued as the granular bands between adjacent membranelles. Henneguy noted that these bands in the membranelles are not broken up into quadrangles, but rather make a continuous line. But, since a comparison of the light bands of the membranelles with those of the body surface shows that the former are wider in comparison with the dark bands, it is probable that the continuous granular band represents the prolongation of only the left side of the "Rippenstreifen" (pl. 17, fig. 29), which is also continuous. On this basis the wider light bands would represent the prolongation of both the "Zwischenstreifen" and the part of the granular band in which the light quadrangular areas cut into the granular zone around the bases of the cilia.

The body stripes are very numerous. On either the dorsal or the ventral surface of the animal as many as 50 to 100 may be counted, indicating a total of between 100 and 200 stripes. According to Kalmus (1929), there are only about 30 ciliary rows on the entire body surface, but this is not in accord with the observations of any other worker.

Entz (1901) describes an anus as a large vacuole at the posterior end, into which are said to lead two long canals. He also describes additional "Neben-vacuoles" in the outer layer of the body surface. But according to the observations of Henneguy (1890b) and Fauré-Fremiet (1912), as well as those of the writer, there is no evidence of a contractile vacuole in *Fabrea*, a condition common to most marine Protozoa.

Kalmus (1929) describes a cytoproct as being situated in the anterior end of the proboscis, in which fecal material is said to agglomerate almost entirely without fluid. A glance at his drawing will show that this fecal mass is simply a collection of the refractive bodies described by Henneguy (1890b). These bodies may be scattered in different regions of the body, but when present in small numbers tend to collect in a mass in the anterior end of the proboscis (pl. 15, fig. 1). Observations made by the writer indicate that they are globules of fat.

The color of *Fabrea salina* varies from a violet blue to a greenish black. None of the forms studied by the writer was yellowish brown, as reported by Kalmus (1929). The color is given by minute pigment granules scattered throughout the ectoplasm and endoplasm. A very complete study of the solubility, absorption spectrum, and chemical properties of this pigment has been made by Donnasson and Fauré-Fremiet, who have given it the name "Fabrein" (Donnasson and Fauré-Fremiet, 1911; Fauré-Fremiet, 1911; Fauré-Fremiet, 1912).

The pigment granules in *Fabrea* are more numerous in the ectoplasm, especially in the "Rippenstreifen." The characteristic black pigment spot near the anterior end of the adoral zone is formed by a mass of these pigment granules. It has not been possible to ascribe any special function to this spot. It may be that the pigment granules are affected in some way by light, and that this is a particularly sensitive area.

Internal characters visible in the living animal.—The endoplasm of *Fabrea* has been described by Fauré-Fremiet (1912) as a heterogeneous system of two fluid parts of different viscosities, the continuous phase corresponding to the hyaloplasm and the vacuoles to the paraplasm of early cytologists. The continuous phase is more viscous, and contains the pigment granules, fat globules, and osmiophilic inclusions. Fauré-Fremiet (1912) describes the vacuoles which fill up the interstices of the reticulum as "vacuoles paraplasmiques." He finds that they readily absorb brilliant cresyl blue, and to a less degree neutral red, because of the great solubility of the cresyl blue in the high concentration of salt in *Fabrea*. The size dimensions vary from 1 or 2 to 4 or 5 μ , but are approximately the same in any one individual. The present investigation supports the observations of Fauré-Fremiet in respect to the affinity of these bodies for brilliant cresyl blue and neutral red. On this basis they are considered to correspond to what has been termed the "vacuome" in Protozoa.

The mitochondria can be seen in the living animal as highly refractile granules scattered throughout the cytoplasm. They stain, as Fauré-Fremiet reports, with dahlia violet. They may also be stained with a weak solution of Janus green B, but often the animals are so darkly pigmented that the mitochondria are obscured.

Lipoid bodies vary greatly in number and distribution, even in individuals from the same culture. They appear in living animals as highly refractive globules, which may be dispersed, especially in the anterior part of the animal, or collected toward the anterior end of the proboscis (pl. 15, fig. 1). These globules were observed by both Henneguy (1890b) and Fauré-Fremiet (1912). Tests for glycogen have proved negative.

The macronucleus of *Fabrea*, if stretched out, would be about two-thirds the length of the animal, from about 100 to 150 μ . A cross section appears as an ellipse of about 8 \times 10 μ . In the living animal it is a clear homogeneous body in which may be seen refractive nucleoli. The granular appearance of the ground substance common to fresh-water ciliates is not apparent. Fauré-Fre-

miet (1912) considers the reason for this to be that in Protozoa living in concentrated salt water the normal state of the chromatin approaches a true solution.

The macronucleus of *Fabrea* was observed by Henneguy (1890b), Fauré-Fremiet (1912), Entz (1901), and Kalmus (1929), but it is highly probable that none of these has seen the micronuclei. Entz (1901) and Kalmus (1929) both report a single micronucleus, which is quite large, according to their drawings. *Fabrea* has several micronuclei, the number varying from one to ten, or rarely up to twenty. They are only from 1 to 2μ in diameter, and cannot be seen in the living animal.

The cytoplasm contains varying numbers of food vacuoles. There is no regularity in their arrangement. They do not seem to follow any set course, but are eventually moved by cytoplasmic movements to the posterior end of the animal.

Movements and feeding activities.—*Fabrea* is very active, spending most of its time swimming and feeding. The course of movement is a spiral one in a forward direction, the body revolving from left to right. At times it may reverse its movements. The cilia beat in metachronous fashion, undulations starting at the anterior end and moving posteriorly and obliquely to the right. The anterior membranelles beat first, the wave continuing rhythmically into the buccal funnel. The stroke of a single membranelle starts on the shorter, right edge and moves to the left. In this way food particles are whipped into the groove leading into the buccal funnel, where they are compressed into food vacuoles which are guided into the endoplasm.

The principal sources of food are small flagellates, particularly *Oxyrrhis marina* and *Dunaliella salina*, and bacteria. But, as reported by Henneguy (1890b) and Fauré-Fremiet (1912), *Fabrea* is often cannibalistic, especially when food is scarce.

Encystation.—Encystation occurs whenever the water in a culture begins to evaporate. Excystation may be induced by placing cysts in fresh culture medium. The length of time required varies according to the age of the cyst. A study of the process of encystation is being made by the writer, and will form the subject of a future paper. A drawing of a young cyst is shown in pl. 15, figure 2.

MORPHOLOGY

ECTOPLASMIC STRUCTURES

Pellicle and ectoplasm.—As observed by both Henneguy (1890b) and Fauré-Fremiet (1912), the pellicle of *Fabrea* is extremely thin and has under it a granular layer of ectoplasm. This layer is only 2 or 3μ in thickness and is filled with granules. Pigment granules are densely packed in the "Rippenstreifen," but are lacking in the "Zwischenstreifen." There are apparently two other types of granules, both of which appear to be chondriosomal in nature. Of these, the smaller, spherical ones are similar to the mitochondria of the endoplasm. These disappear after the use of fixatives which do not ordinarily pre-

serve mitochondria. The other granules, however, are larger, more rodlike, and may often remain after fixation in such fixatives as Schaudinn's with acetic acid. They may then be stained with iron haematoxylin. They also show a strong affinity for osmic acid. But in the Regaud and Champy-Kull techniques they stain along with the spherical mitochondria. Similar ectoplasmic granules with an affinity for mitochondrial stains are described by Peshkowskaya (1928) in *Climacostomum virens*, and by Studitsky (1930) in *Dileptus gigas*. Both of these observers conclude that these granules are of a mitochondrial nature. Causey (1926) finds that in *Paramecium* there are both granular and rod-shaped mitochondrial elements, and that some of these may show a remarkable persistence in fixatives containing acetic acid.

As observed by Henneguy (1890b), there is no distinct alveolar layer in *Fabrea*. The nearest approach to one is found underneath the adoral zone, where there is a thick vacuolated ectoplasmic layer between the basal lamellae of the membranelles.

Cilia.—The basal granules of the cilia are very difficult to distinguish from the other granules which fill the ectoplasm. In favorable osmic preparations, and sometimes in Mallory or Yabroff sections, they may be seen just under the pellicle as rows of granules connected by fine longitudinal fibrils. No transverse connections between ciliary rows, and no basal ciliary rootlets extending into the endoplasm, have been observed. The distance between basal granules of cilia in the same row is about 1μ , and that between adjacent rows in the center of the animal about 1.5μ .

The peristomial field.—According to the observations of Henneguy (1890b), there is present below the adoral zone of *Fabrea salina* a "lame basilaire adorale," consisting of a continuous plate or a series of parallel fibrils constituting the base of the adoral crest. Each membranelle is said to form a basal lamella which is inserted in the basal plate (text fig. A, 1f). Henneguy states that he was unable to find a "Basalfibrille" (Schuberg, 1890), but that, since the basal lamella is inserted on the basal plate along its whole length instead of being triangular as in *Stentor*, the "lame basilaire adorale" of *Fabrea* corresponds to the "Basalfibrille" of *Stentor*. Fauré-Fremiet (1912) seems to agree with these observations.

The present investigation does not support the conclusions of Henneguy (1890b) and Fauré-Fremiet (1912) that the "ligne à double contour" beneath the ectoplasm of the adoral zone constitutes a basal plate. Almost every section shows an *apparent* basal plate. But with careful focusing the "continuous line" always resolves itself into separate basal lamellae that undergo extreme torsion, thus giving an optical illusion of an apparent fusion to form a continuous line. In sections it is possible to see the basal lamellae ending freely in the cytoplasm (pl. 15, fig. 3), and a study of serial sections through the same animal shows no trace of a connecting plate. These observations support the conclusions of Dierks (1926), who maintains that the so-called "Basal-band" or "Basalfibrille" of *Stentor* is likewise the result of an optical illusion.

In *Fabrea* it is not difficult to see the double rows of basal granules of the

two rows of cilia making up the membranelles. At times they are so closely set together that they appear as solid lines. This appearance is interpreted by the writer as the fusion of basal granules rather than as a "Doppelbalken" (Gelei, 1926). Below the basal granules the ciliary rootlets seem to fuse into a single plate, the basal lamella. Bishop (1927) describes a similar basal lamella in *Spirostomum ambiguum*, but states that she has never been able to observe any evidence of fibrillar structure in it. In *Fabrea* the fibrillar nature is usually quite evident. Sections cutting across a lamella show definite fibrils, which are in a single row (pl. 17, fig. 25). A surface view of a lamella shows not only the fibrillar structure, but also the granular enlargements of the fibrils where they end on the lower base of the lamella (pl. 15, figs. 8, 9). In sections cut parallel to the horizontal surface of the adoral zone, the basal lamellae can be followed until they disappear into the endoplasm, and there is no evidence that a fusion occurs (pl. 17, fig. 25). In the spaces between the lamellae are found the "alveoles" described by Henneguy (1890b) and the mitochondria described by Fauré-Fremiet (1912).

The neuromotor system.—Since the description of the neuromotor system of *Diplodinium* (= *Epidinium* Crawley, 1923) *ecaudatum* by Sharp (1914), and of *Euplotes patella* by Yocum (1918), similar systems have been described in many ciliates. An intricate pellicular pattern has been revealed in many genera by the silver technique of Klein (1926), Chatton and Lwoff (1930), and Gelei and Horvath (1931). Evidence that the internal "conducting" and external "supporting" fibrils are intimately connected has been given by Lund (1933) in *Paramecium multimicronucleata* and by Turner (1933) in *Euplotes patella*.

In *Fabrea salina* it has not been possible, by any of the silver methods, to demonstrate a pellicular pattern. Silver techniques have, however, been valuable for study of ciliary arrangement and connecting fibrils. Text figure A, 2, represents a schematic diagram of the fibrillar system, based upon studies of silver, osmic, iron haematoxylin, and Mallory preparations. This system, which will be designated as the neuromotor system, may be divided into five parts: (1) the body cilia, (2) the longitudinal fibrils connecting cilia in the same rows, (3) the adoral zone of membranelles, (4) the adoral fibril, and (5) the neuromotorium.

The body cilia and connecting longitudinal fibrils have already been described. The fibrils are continuous on the dorsal surface, but on the ventral surface are interrupted by the coiling adoral zone. Each fibril of the dorsal and ventral surfaces, with the exception of those that merge with one another, is connected at the adoral zone with the basal lamella of a membranelle. The basal lamella is connected by fibrils running across the peristomial groove with a granule on the adoral fibril (pl. 15, figs. 8, 9; text fig. A, 2). The adoral fibril starts at the anterior tip and follows the course of the inner border of the adoral zone, from which it is separated by the peristomial band ("Randstreifen" of Maier, 1903). Along this fibril there are closely set granules, which form basal bodies of the peristomial ciliary row, and which are connected to

the basal fibrils of the membranelles. The peristomial fibril is continued beyond the end of the adoral zone on the wall of the funnel, and ends in a ganglion-like body on the left wall of the ventral lobe. This body, from 2 to 3μ in diameter, is considered to be homologous with the motorium described by other writers. It can be demonstrated as a black body with iron haematoxylin or silver nitrate. After Mallory's triple stain it appears red (pl. 17, figs. 24, 31). It lies 2 or 3μ from the surface, and can in no way be considered a skeletal element. From the motorium arise several fibrils. The most posterior one is the adoral fibril, which follows the course of the adoral zone to the anterior tip of the animal. Anterior fibrils have been carefully traced to determine if there is a circumpharyngeal fibril uniting with the adoral fibril where it leaves the buccal pouch. The wall of the ventral lobe is so granular, however, that it has been impossible to trace the most anterior fibril far enough to see if there is such a connection. The other fibrils, of which there are two or three, appear to end blindly in the endoplasm of the ventral lobe.

The longitudinal fibrils of the anterior ventral surface connect the cilia of the frontal field. Some of these fibrils are connected posteriorly with the outer border of the membranelle zone. Those on the left curve around the buccal pouch to the ventral lobe, where they are continued on its anterior wall to the floor of the cytostomal area. Anteriorly the fibrils of the frontal field tend to converge, and end very obliquely on the adoral fibril (text fig. A, 2).

The neuromotor system of *Fabrea* is very much like that described by Campbell (1926) in *Tintinnopsis nucula*. A definite circumesophageal ring occurs in *T. nucula*, and in *Balantidium coli* (McDonald, 1922). No connection between somatic cilia and the motorium could be demonstrated by Campbell (1926) in *T. nucula*. In *Fabrea salina*, however, the longitudinal rows of body cilia are directly continuous with the membranelles, and through them with the adoral fibril. The fact that the entire body surface of *Fabrea* is covered with cilia would probably account for this difference. The connection of the adoral fibril of *T. nucula* with the membranelle zone by fibrils crossing the peristome is identical with that in *Fabrea*, where the fibrils cross the peristomial band.

ENDOPLASMIC STRUCTURES

The endoplasm of *Fabrea* is continuous with the ectoplasm, there being no evidence of a cortical lamella (Wetzel, 1925), or boundary layer (Sharp, 1914; McDonald, 1922).

Mitochondria.—Distributed throughout the continuous phase of the endoplasm are numerous mitochondria similar to the granular types described by Causey (1926). It is probable that the larger ectoplasmic granules, which behave like mitochondria in every way except for their persistence in "mitochondrial solvents," are comparable to the rodlike mitochondria also described by Causey (1926) in *Paramecium*. Both types of granules blacken in Mann-Kopsch and Kolatchev preparations, but they can be bleached and then stained red with Champy-Kull technique (pl. 17, fig. 23).

Fat.—The distribution of fat globules has been described. Fauré-Fremiet (1912) states that they are soluble in alcohol, stainable with Sudan III, that they reduce osmic acid, and do not absorb aniline dyes—results verified in the present study. The globules vary in size from about 1 to 3μ . After formalin or Carnoy fixation they stain readily with Sudan III (pl. 17, fig. 32). They blacken with osmic acid, but can be bleached readily with turpentine.

Golgi apparatus.—There are other osmiophilic bodies which resist prolonged bleaching in turpentine, and which are apparent after the fat has been bleached with hydrogen peroxide. These are in the form of discrete spherical bodies, from 1 to 2μ in diameter, and are often characterized by an osmiophobic center and an osmiophilic rim (pl. 17, fig. 28). They are similar to the structures described as Golgi material in other Protozoa (Hirschler, 1927; Bush, 1934). Granules of similar size and distribution are revealed by the Yabroff (1928) modification of the Da Fano silver method (pl. 17, fig. 27). In osmic preparations, after sections have been bleached in turpentine from one to ten days these bodies are still apparent. They are distributed throughout the cytoplasm, but are more abundant in the outer part of the endoplasm and in the central and posterior regions of the body.

The vacuome.—Hall (1931) has described osmiophilic bodies in *Stylonychia* which he considers to be identical with granules that stain with neutral red. On this basis he concludes that the osmiophilic bodies in Protozoa and the vacuome are the same structures. A study of the effects of neutral red in *Fabrea* does not support this conclusion. Hall states that in *Stylonychia* "the description of the vacuome has been based primarily on observations made during the first 20–30 minutes after the preparation has been set up. This precaution was taken in order to avoid, so far as possible, the confusion of 'preëxisting vacuome with other inclusions which might be 'induced' by prolonged exposure to neutral red."

With this same procedure, it is the "paraplasmic globules" of *Fabrea* which show an affinity for neutral red, and also for brilliant cresyl blue, which, according to Hall (1931), is also a stain for the vacuome. Food vacuoles will also take up these dyes. The osmiophilic bodies, however, do not begin to stain until signs of degeneration have begun to appear.

On the basis of these observations, the conclusion of the writer is that in *Fabrea salina* there are two separate groups of cytoplasmic inclusions which correspond to the structures described as the vacuome and Golgi apparatus in other Protozoa. Bush (1934) and MacLennan (1933) have found similar results in ciliates of such widely separated groups as the Haptophryidae and the Ophryoscolecidae, and Baker (1933) has given conclusive evidence that the same holds true for *Euglena gracilis* Klebs.

The macronucleus.—The macronucleus is band-formed, usually twisted into a C shape, and extends from the proboscis almost to the posterior end of the animal (pl. 16, fig. 10). The nuclear membrane is difficult to distinguish, even in fixed and stained material, except where there has been some shrinkage of the nuclear contents. At such times the membrane may be visible.

The appearance of the nucleus in stained preparations depends on both the method of fixation and the physiological condition of the animal. In the typical resting condition, after either Bouin or Schaudinn fixation followed by iron haematoxylin, it shows a mass of very small chromatin granules among which are scattered irregularly shaped bodies of 1μ or less in length (pl. 16, fig. 10). There is little or no evidence of a reticular structure, although the chromatic granules often have a streaming appearance.

Nucleoli have been observed in the nuclei of most ciliates, in which they always stain heavily with iron haematoxylin. These bodies have been called nucleoli, Binnenkörper, macrosomes, and other terms. The words endosome and Binnenkörper are often used to designate single large intranuclear bodies, and macrosome and microsome suggests that the difference between the large and small granules is only one of size. Collin (1912) considers the macrosomes as true nucleoli and the microsomes as true chromatin granules. The larger granules will be referred to as nucleoli when mentioned hereafter in the present discussion.

Zinger (1929) gives a detailed description of the structure and cyclical changes of the nucleoli in ciliates. He concludes that the larger bodies are of nucleolar substance, and that the fine granules are of chromatin.

In *Fabrea* fixed with Bouin's or Schaudinn's fluids followed by the Feulgen method, chromatin granules appear red or violet. With Schaudinn fixation, followed by Feulgen, vacuoles are noticeable (pl. 16, figs. 16, 17). It is probable that these are owing, as Calkins (1930a) found in *Uroleptus halseyi*, to the fact that the nucleoli have been hydrolyzed, leaving clear spaces which indicate their former position. In animals from old cultures, however, and in giant forms, the nucleoli are still visible as refractive bodies, but they do not stain (pl. 16, fig. 19).

After Flemming fixation followed by iron haematoxylin, granular strands of chromatin appear, running parallel to the longitudinal axis of the nucleus, but with many cross connections. Nucleoli collect in the nodes of the apparent reticulum (pl. 16, fig. 20). After Champy fixation the nucleus may show a reticular aspect similar to that produced by Flemming's fluid. Large vacuoles appear after fixation with sublimate-acetic in 95 per cent alcohol, with nucleoli scattered in the network (pl. 16, fig. 22). A similar alveolar structure is reported by Turner (1930) in *Euplotes patella* after the same method of fixation.

With Schaudinn fixation and the Borrel stain, the macronucleus shows a dense mass of red chromatin granules among which are scattered the nucleoli, the latter taking the green stain (pl. 16, fig. 18). However, if the slides are left too long in the aniline blue-picric acid mixture, the chromatin granules may stain green. Similar results with this stain have been reported by Calkins (1930a).

Comparisons of different aspects of the macronucleus of *Fabrea* after various methods of fixation indicate that the chromatin granules are free in the nucleoplasm, as considered by Greenwood (1896) to be the condition in *Car-*

chesium polypinum. The apparent reticulum seems to be a result simply of the method of fixation.

The present tendency is to restrict the use of the term chromatin to substances containing thymonucleic acid, and the Feulgen reaction is regarded as specific for this substance. On this basis the fine granules may be said to represent the true chromatin in the macronucleus of *Fabrea*. The nucleoli, since they do not stain by this method, are not to be considered as large chromatin masses.

The micronuclei.—In the resting stage, micronuclei are exceedingly difficult to find in *Fabrea* except in sectioned material. In whole mounts the cytoplasm shows such an affinity for nuclear stains that differentiation for micronuclei is almost impossible. They are hardly ever visible with the Feulgen stain, but they have a great affinity for iron haematoxylin. The number of micronuclei varies from one to ten, four to eight being the most common.

Rarely there may be less than four, or more than ten. With any type of nuclear stain, each nucleus appears as a homogeneous sphere from 1 to 1.5μ in diameter, surrounded by a definite nuclear membrane. Between the membrane and the central chromatin mass is a clear space which is probably caused by shrinkage. It is always present, however, and is of value in distinguishing the micronuclei from other basophilic granules in the cytoplasm. The diameter of the nucleus, including the halo and the nuclear membrane, is rarely over 2μ . There is no evidence of a granular structure in the resting nucleus (pl. 18, fig. 33).

DIVISION

Cytoplasmic structures.—The process of binary fission in *Fabrea* follows the usual type of transverse division characteristic of ciliates. The first indication of approaching division is the elongation of the posterior part of the body and the cessation of feeding. The first sign of a new adoral zone is the appearance of a refractive longitudinal band below the old adoral zone. Sections of this band show it to be a homogeneous layer filled with small granules, among which basal bodies of cilia are beginning to appear (pl. 17, fig. 26). Almost immediately the membranelles begin to form. They are at first very short but of normal width. The cilia are separate but soon fuse into short plates. The movements at this time are quite irregular, as noted by Bishop (1923) in *Spirostomum ambiguum*.

It is some time before the formation of basal lamellae is completed. At first only the anterior part of the adoral zone is present, but the posterior region is indicated by proliferation of the granular band posteriorly and to the right (pl. 16, fig. 12). The new adoral zone forms not directly below the old, but toward the right (pl. 16, figs. 11, 12).

Henneguy (1890b) noted that the old peristomial excavation becomes partly effaced and the adoral zone almost superficial. He states that at a more advanced stage of division, after the new adoral zone of the posterior individual is completed, the old one returns to its original form as the adoral zone of the anterior individual. According to the observations of the writer, the old

membranellae of the adoral zone are dedifferentiated and new ones formed. The development of new membranellae just within the old ones occurs simultaneously with the absorption of the old adoral zone, as described by Calkins (1912) in *Blepharisma undulans*, and Schmähl (1926) in *Bursaria truncatella*. In sections the new adoral zone appears granular, like that of the developing zone of the posterior daughter, and the old zone begins to show disintegration of the basal lamellae (pl. 17, fig. 26). There is no period of rest, as described by Kalmus (1929), the dividing animals continuing to swim about rapidly until separation occurs.

The old peristomial cavity of *Fabrea* is entirely eliminated in division, and a new cavity is formed after the appearance of the new adoral zone. A similar cavity is formed in the posterior daughter by the sinking in of a groove which forms on the right side of the developing adoral zone. During this stage profound internal changes are taking place. There are streaming movements of the cytoplasm from the nucleus toward the ends of the animal (pl. 17, fig. 26).

Division of the macronucleus.—As the time for division approaches, nucleoli become more prominent in the macronucleus (pl. 16, fig. 10). The nucleus then begins to contract. It becomes increasingly shorter and thicker, and shows a clear zone between the chromatin mass and the nuclear membrane (pl. 16, fig. 11). This clear region is not apparent except in stained material, which indicates a shrinkage in fixation. But, since the normal vegetative animals on the same slide show no such halo, it is evident that a change has taken place.

In the living animals the nucleus at this time appears amoeboid, showing pronounced contractions and changes of shape. Finally it becomes ovoid, but even at this stage sections give evidence of internal streaming movements (pl. 17, fig. 26). Although nucleoli are visible in sectioned material, in whole mounts the nucleus appears homogeneous (pl. 16, figs. 11, 12).

Only after the new adoral zones are almost completely formed does the nucleus begin to elongate (pl. 16, fig. 13). The most noticeable change in the elongating nucleus is the absence of nucleoli. They seem to have been either dissolved or thrown out into the cytoplasm during the amoeboid contractions of the nucleus. Evidence is often found in sectioned nuclei that material is being cast out into the cytoplasm. During division the cytoplasm around the nucleus becomes very dark. Slides stained with iron haematoxylin show clouds of fine granules, as well as large irregular ones (pl. 16, fig. 12). The nature of these granules is not understood. At times they are so dense that it is almost impossible to differentiate the macronuclear details except in sectioned animals. The most striking fact is that the larger granules often stain dark red with the Feulgen reaction, indicating the presence of nucleic acid. This leads to the conclusion that there is a casting out of chromatin material, and possibly also of nucleoli, during the contraction of the macronucleus. A similar casting out of materials seems to occur during the constriction of the macronucleus into ovoid bodies at the beginning of conjugation, and again in the development of the new macronuclei after conjugation. Such a process of chromatin extrusion has been described by Calkins (1912; 1919), Diller

(1928), Kidder (1933a; 1933c), and Wenrich (1926) in other ciliates.

In reference to the nucleoli ("X" granules) of *Uroleptus halseyi*, Calkins (1930a) states that "what they are is problematical but they form part of the substance of the nucleus that is consigned to the cytoplasm at the time of division. With them about one-third of the entire chromatin content of the macronucleus is likewise shed into the cytoplasm, always with a loss of the constituent nucleic acid."

Causey (1926) thinks that the highly stainable bodies that collect in the cytoplasm of ciliates during these stages may be masses of mitochondria fused by imperfect fixation. It is possible that this is true of these inclusions in *Fabrea*. They do migrate to the periphery, and later disappear from this region, as Poljansky (1934) describes also in *Bursaria*. The fact that they take chromatin stains, including the Feulgen stain, suggests that they are chromatin derivatives, but it must be realized that some granules of mitochondrial nature are also preserved by the so-called mitochondrial solvents and stained by haematoxylin.

The elongating macronucleus of *Fabrea* is rod-shaped (pl. 16, fig. 14). As it begins to constrict in the center, the distal ends become slightly larger in diameter. The chromatin granules may be in rows, as if streaming movements were occurring (pl. 16, fig. 15). The nucleus elongates slowly, finally pulling out the nuclear membrane as a thread which soon breaks (pl. 16, fig. 15). There is never any indication of the discarding of nuclear material between the halves as the nucleus pulls apart.

There is nothing in the dividing macronucleus of *Fabrea* that is comparable to the "Kernspalt" of hypotrichs. It is probable, however, that a complete reorganization of nuclear contents, comparable to that described by Turner (1930) in *Euplotes patella*, occurs during the contracted phase, because the elongating macronucleus after this contraction is markedly different in appearance. Only rarely are nucleoli present after division, and these are small and rodlike.

Division of the micronuclei.—The changes in the micronuclei in division are difficult to determine, because of their extremely minute size and their loss of affinity for nuclear stains during division. In a resting state they appear as homogeneous, darkly stained ovoid bodies from 1 to 2μ in diameter (pl. 16, fig. 10; pl. 18, fig. 33), which are often closely applied to the macronucleus. As soon as the macronucleus begins to contract, the micronuclei move away from their position in contact with it, but remain within a radius of from 5 to 20μ . They now show signs of vacuolization and swelling (pl. 18, fig. 34). This continues until the nuclei reach a diameter of about 3μ . At this time there is apparently a reticulum (pl. 18, fig. 35), although the entire lack of chromophilic substance makes this point difficult to determine. The next stage that can be made out with any degree of certainty is a definite spindle with a very large number of granules in the center (pl. 18, fig. 36). The spindle poles do not appear to end in a common point, and no centrioles or polar cones (Bishop, 1925) are evident. The size of the spindle is about $2.5 \times 5\mu$.

By the time the macronucleus has reached its greatest contraction (pl. 16, fig. 12), the chromatin granules of the micronucleus have migrated to the poles of the spindle and are difficult to detect. The spindle fibers, however, are refractive, and can be distinguished more easily (pl. 18, fig. 37). As the granules fuse they again become more basophilic (pl. 18, fig. 38). The spindle draws out until the daughter nuclei are from 15 to 20 μ apart (pl. 18, fig. 39). These spindles lie in all directions, as they do in *Spirostomum ambiguum* (Bishop, 1925). The connecting strand then breaks, but may remain attached for some time to the daughter nuclei (pl. 18, fig. 40).

By the time that the constriction of the cytoplasm has started, the daughter micronuclei have completely reorganized into homogeneous spheres about 1.5 μ in diameter, and may be seen attached along the edge of the elongating macronucleus (pl. 16, fig. 14). The number of micronuclei going to each daughter animal depends entirely on their position along the macronucleus.

The division of the micronuclei is always synchronous in *Fabrea*, although at times one or more of the nuclei may not divide. This is especially true of the nuclei that are at some distance from the macronucleus (pl. 16, fig. 12). At no time in the process is it possible to count the number of chromosomes, if the chromatin granules on the spindle may be considered as such. The small size of the nucleus and the large number of granules also make it impossible to determine the method of division of the chromosomes (pl. 16, fig. 11; pl. 18, fig. 36). No intrademes or centrioles are visible in division nuclei, although they seem to be present in some stages of conjugation.

Final stages of division.—After the formation of the adoral zone of the posterior daughter, a circular constriction develops. The connecting part is gradually reduced to a small stalk. As a result of revolutions at different speeds, and of definite bending movements, especially by the anterior daughter, the connection is soon broken. The entire process of separation, from the first visible signs to final separation of daughter animals, requires from three to four hours. The greater part of this time is taken up by the earlier stages, only from 30 to 45 minutes being required for separation after the constriction plane is formed.

CONJUGATION

CYTOPLASMIC STRUCTURES

Conjugation epidemics of *Fabrea* are always preceded by rapid divisions, with the result that the conjugants are smaller than normal vegetative individuals. They are at first more transparent than vegetative forms, as noted by Prandtl (1906) in *Didinium nasutum*. This transparency is rapidly lost after the union of the conjugants, owing to the appearance of clouds of dark granules around the micronucleus (text fig. B, 1). Conjugation may be between individuals of the same size or of different sizes, but there is never a marked difference.

Conjugants fuse along the left ventral surfaces of the frontal field in the region anterior to the sinking in of the peristomial area. This causes them to

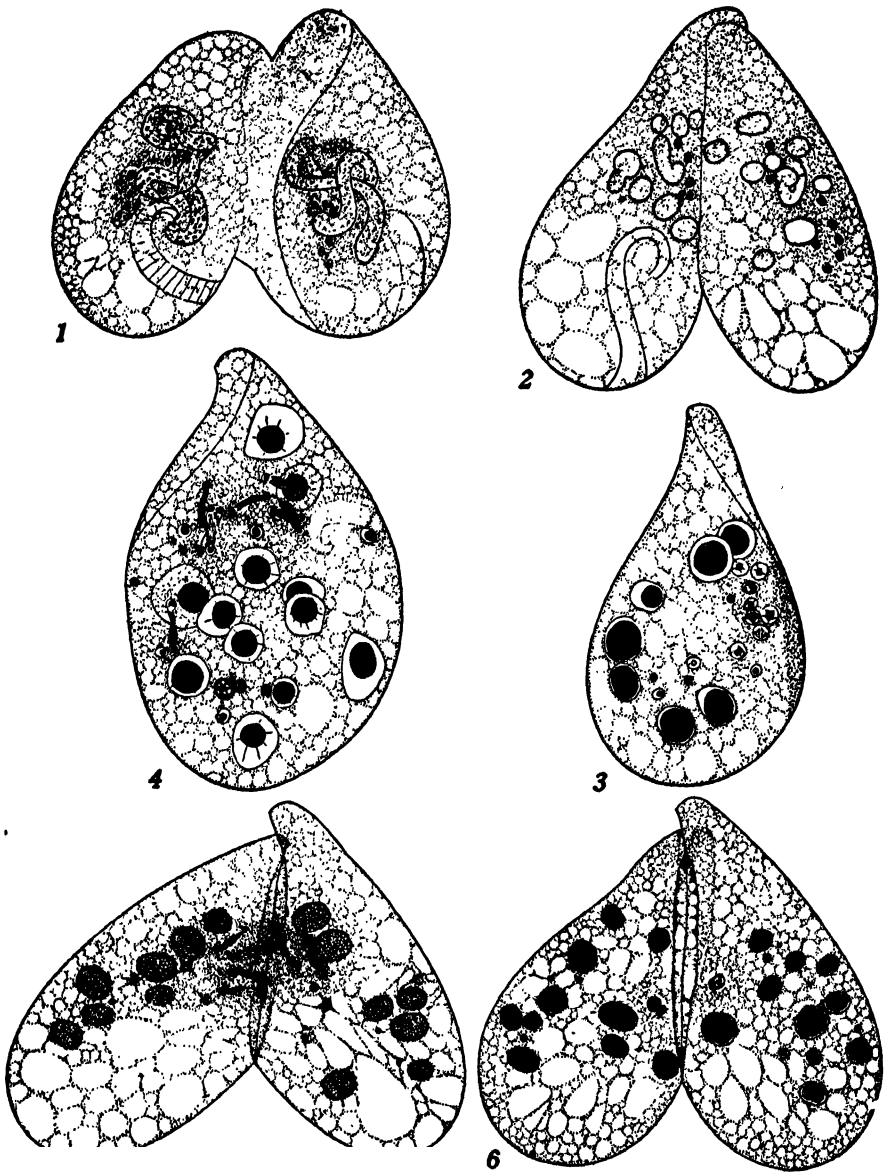


Fig. B. Stages in Conjugation. All drawings $\times 400$.

1. Conjugants at first maturation division. The macronucleus is beginning to constrict. Schaudinn, Heidenhain.
2. Second maturation division. Macronucleus breaking into oval bodies. Schaudinn, Feulgen.
3. Single member conjugating pair in second maturation division, showing some degenerating micronuclei. Schaudinn, Heidenhain.
4. Same, in telophase of second maturation division. Schaudinn, Delafield.
5. Telophase of third maturation division. Schaudinn, Heidenhain.
6. Fusion of pronuclei. Schaudinn, Heidenhain.

face in opposite directions. A granular protoplasmic bridge is formed between the conjugants, but no endoplasmic connection occurs until after fertilization. Isolated conjugating pairs may remain attached up to 48 hours before separation. Pairs which have not separated by that time seem to be abnormal, because they remain permanently fused and die after swimming around for a few days.

There is no union of oral regions (text fig. B, 1, 2), but there is evidence that some dedifferentiation of the peristomial region may occur, as is said to be true in *Bursaria truncatella* (Prowazek, 1899; Poljansky, 1934), *Chilodon uncinatus* (MacDougall, 1925), and *Euplotes patella* (Turner, 1930). Although the adoral zone does not appear to undergo any change, there is apparently a complete closure of the buccal cavity. There is often a large vacuole anterior to the inturned adoral zone. This seems to push against the anterior wall of the buccal pouch and press it against the posterior wall. Most food vacuoles disappear, but the conjugants remain active, swimming around throughout the entire processes of conjugation and reorganization.

NUCLEAR PHENOMENA

Degeneration of the old macronucleus.—Early in conjugation the macronucleus begins to contract toward the center of the cell. It becomes very active, exhibiting twisting and writhing movements as if it were trying to tie itself into knots. At this time the larger nuclear granules begin to disappear, as they do in division, and a cloud of granular inclusions collects around the nucleus (text fig. B, 1). The micronuclei collect in this granular zone and undergo the first maturation division. This stage seems to last for a long time, since it is one of the most common found in mass preparations.

The macronucleus then begins to constrict into separate oval or spherical bodies (text fig. B, 2). The number of these varies from 4 to 20, but the most common number is either 8 or 10. They appear to consist entirely of large chromatin granules embedded in a plastin matrix (text fig. B, 3, 4). Sections, however, show that a few nucleoli are still present (pl. 19, fig. 61). At this stage the micronuclei undergo the second maturation division. The nuclear contents are contracted away from the membrane, and the latter is then clearly visible.

At the time of the third maturation division in the micronuclei, the nuclear fragments begin to show vacuoles, with the chromatin granules collected on the nodes of the reticulum (pl. 19, figs. 62–65; text fig. B, 5). These granules become more prominent (text fig. B, 6) and begin to fuse into irregular masses (pl. 19, fig. 65). The vacuoles increase in size and often appear to burst (pl. 19, fig. 64) in a manner similar to that described by Bishop (1923) in *Spirostomum ambiguum*. The fragments stain intensely with all nuclear stains, but gradually lose their staining capacity and are absorbed (text fig. C, 1–3). They simply fade out into the cytoplasm, and are not thrown out of the cell—as has been described, but not verified, in some ciliates. It is probable, as Calkins (1930a) points out, that the nucleic acid given off from the

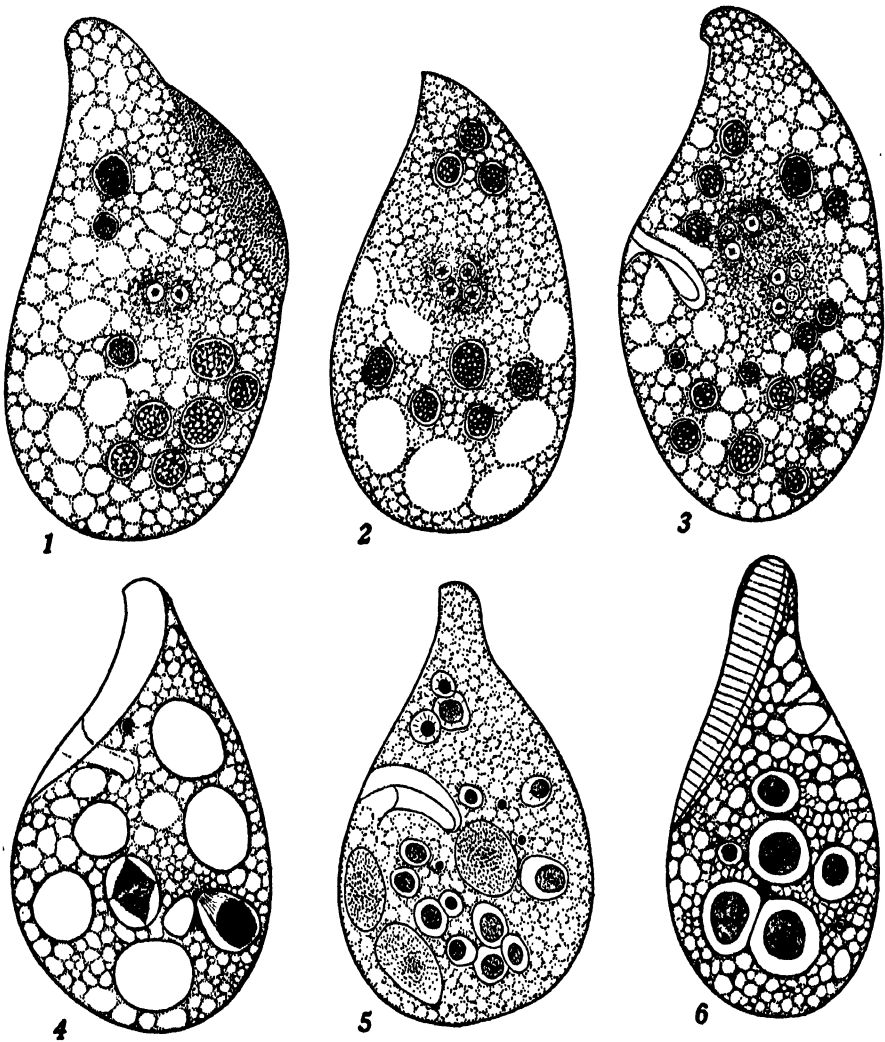


Fig. C. Conjugation and reorganization. All drawings from Schaudinn-Heidenhain preparations. $\times 500$.

1. Single member conjugating pair after first amphinuclear division.
2. Same, after second amphinuclear division.
3. Same, after third amphinuclear division, showing differentiation of new macronuclei and micronuclei.
4. Section of exconjugant, showing basophilic granules in developing macronuclei.
5. Exconjugant containing fourteen developing macronuclei at the time of their loss of staining capacity.
6. Exconjugant with five developing macronuclei, showing formation of basophilic bodies.

degenerating old macronucleus is incorporated into the developing new macronuclei, as is indicated by their gradual increase in staining capacity.

The first maturation division.—The first maturation division is completed in the period when the macronucleus contracts and constricts into separate spheres. There is no evidence of a preliminary division, such as occurs in *Euplotes patella* (Turner, 1930), or of a progamic division, as is reported to occur in various parasitic ciliates (Dogiel, 1925). The number of nuclei taking part in this division varies according to the number in the vegetative state. From 8 to 20 first-maturation nuclei have been counted in serial sections, indicating that the usual number may have been increased before conjugation.

Usually all the micronuclei undergo the first maturation division, and since the divisions are not always synchronous, several different stages may be found in the same animal. At times nuclei may be found which do not divide. As in binary fission, these are nuclei at some distance from the macronucleus (text fig. B, 1).

The first maturation division in *Fabrea* is of the so-called parachute or candelabra type. After moving into the dark granular zone around the macronucleus, the micronuclei begin to swell, and almost completely lose their affinity for nuclear stains of any kind. The nucleus becomes granular, and small vacuoles begin to appear. A clear space separates the chromatin mass from the nuclear membrane (pl. 18, fig. 41). The nucleus in this stage is about 1.5μ in diameter. It begins to swell rapidly and may reach a diameter of 3 or 4μ . At this time there appears a reticulum upon which a few granules are scattered (pl. 18, fig. 42). As the reticulum increases in size, the granular nature is less apparent (pl. 18, fig. 43). This is considered to be a later stage than the preceding, since there is a definite increase in the size of the reticulum. A still later prophase stage is represented in plate 18, figure 44. Nuclei of this type are fairly common. A granular network is confined to one side of the nucleus, and a definite granule can be seen on the opposite side. A thread, which is darker than the other fibers of the spindle, leads from this granule to the opposite pole. At this pole the reticulum is so dense that it is impossible to demonstrate a polar granule.

The next observable change is the breaking up of the reticulum into definite granules collected at one pole (pl. 18, fig. 45). These granules then migrate to the equator of the spindle (pl. 18, fig. 46). The chromatin granules on the equator appear to be arranged only on the peripheral fibers of the spindle (pl. 18, fig. 46).

It is impossible to determine the number of chromosomes in the micronucleus of *Fabrea*. In order to estimate the approximate number, separate camera lucida drawings were made at different optical levels, and the numbers of granules on the sketches added, after the method of Calkins (1919). The numbers counted in this way in the anaphase of the first maturation division indicated approximately 48 granules going toward each pole (pl. 18, fig. 46), but the granules are so small that an accurate count is impossible. The telo-

phase stage is barrel-shaped, with an irregular mass of granules at each pole (pl. 18, fig. 47). No later stages in the constriction of the nuclei have been found.

The granules of the prophase spindle are not considered by the writer to represent true chromosomes. They seem rather to correspond to the chromomeres (*chromioles*, Calkins, 1930b) which are divided into two groups in the first maturation division of many ciliates. In *Uroleptus mobilis* (Calkins, 1919), there are about 24 chromioles on the spindle, which pass without dividing to the two daughter nuclei. In *Uroleptus halseyi* (Calkins, 1930b), of 48 granules, 24 go to each pole. In *Euplotes patella* (Turner, 1930) there are 32, with 16 going to each pole. In *Conchophthirius mytili* (Kidder, 1933b), there is a sorting out of 32 chromomeres into two groups of 16 each. These are not considered to be reduction divisions, because, in each, true chromosomes form in the second division and reduction occurs.

The second maturation division.—As is true in most other ciliates, second division stages are extremely rare in *Fabrea*. These can be distinguished from those of the first maturation by the fact that only from 8 to 12 nuclei take part, and degenerating nuclei may be visible (text fig. B, 2, 3). The macronucleus has also broken up into characteristic oval bodies. In contrast to the first maturation division, the second and third divisions appear to occur synchronously in all dividing nuclei.

There appears to be no resting stage after the first division telophase, the second-division spindles forming very quickly. On this spindle are approximately 24 rodlike bodies which are considered to represent true chromosomes formed by fusion of the granules of the first-division spindle (pl. 18, fig. 48). A similar situation is described by Calkins (1919) in *Uroleptus mobilis*, and by Turner (1930) in *Euplotes patella*, in each of which 8 chromosomes are formed.

In the anaphase of the second division there are approximately 12 rodlike chromosomes at each pole (pl. 18, fig. 49). This would seem to indicate a true reduction, as has been reported to occur in most ciliates at this stage. In contrast to the earlier stages, telophases of the second division are among the most common stages encountered, indicating that this is a period of long duration. The spindle, which shows marked torsion, is drawn out to a length of about 15μ , with heavily staining masses of chromatin at the ends (text fig. B, 4; pl. 18, fig. 50). A definite intradesmose is indicated by a darker central fiber.

The third maturation division.—The third maturation division occurs immediately after the second, and can be distinguished from the former in several ways. The spindles are similar except that those of the third division are narrower and more pointed (pl. 18, fig. 51). A similar difference was noted by Manwell (1928) in *Pleurotricha lanceolata* and by Gregory (1923) in *Oxytricha fallax*. The spindles are situated close to the line of union of the conjugants (text fig. B, 5), in contrast to the centrally placed spindles of the second division.

The chromosomes of the third division, as well as those of the second, are almost entirely lacking in basophilic substance. They do not stain deeply with the Feulgen reaction, as do those of many ciliates, but appear as very faint pink rods along the peripheral fibers of the spindle (pl. 18, figs. 49, 51). The number of chromosomes in the third-division anaphase, as in the second division, appears to be 12 at each pole, although their lack of affinity for any nuclear stains makes it impossible to regard this as more than an estimate. Since no metaphase stages have been observed, it is impossible to state whether division of the chromosomes is longitudinal or transverse.

The third-division spindle is drawn out in a manner similar to that of the second division, although an intradesmose is less evident. The chromatin at the ends of the spindles condenses into exceedingly small pronuclei (pl. 18, fig. 52). There may be several pronuclei touching the line of union between conjugants (text fig. B, 5).

Fusion of pronuclei.—The third maturation division results in several very small compact nuclei, from 1 to 2μ in diameter. The actual penetration of the membrane separating conjugants, and the migration toward the stationary nucleus, must occur very rapidly, for such stages have not been observed.

It is apparent from all conjugants which show pronuclei in contact that only one male and one female pronucleus in each individual is functional. Although the third-maturation spindles do not seem to be heteropolar, one pronucleus is always larger than the other at the time of fusion (text fig. B, 6; pl. 18, fig. 53). The larger one is regarded as the female pronucleus. Since migration of the male pronucleus has not been observed, it is impossible to state whether an attraction sphere accompanies it, as occurs in *Didinium nasutum* (Prandtl, 1906), *Uroleptus mobilis* (Calkins, 1919), and *U. halseyi* (Calkins, 1930b). There is, however, a dense granular zone around the fusing pronuclei (pl. 18, fig. 53), and this remains until after the third amphinuclear division. Degenerating pronuclei which do not take part in fertilization rapidly disappear.

The pronuclei fuse in a vesicular rather than in a spindle stage. The chromatin of the female pronucleus spins out into a reticulum while that of the male nucleus is just beginning to break up into granules (pl. 18, fig. 53). Whether the pronuclei form separate spindles during actual fusion has not been determined. By the time the synkaryon spindle forms, however, there is no distinction between male and female elements.

Amphinuclear divisions.—The divisions which follow fertilization occur very rapidly, and are difficult to find. There is, as in *Uroleptus halseyi* (Calkins, 1930b), no apparent resting stage between these divisions. The synkaryon spindle is from 4 to 5μ in diameter, and is distinctly barrel-shaped, with polar caps similar to those described by Manwell (1928) in *Pleurotricha lanceolata*. The chromosomes are ragged and granular, and extend from the pole along the periphery of the spindle (pl. 18, figs. 54, 55). The number of granules appears to be approximately the same as that of the first maturation division. The telophase stage is drawn out with a typical connecting strand

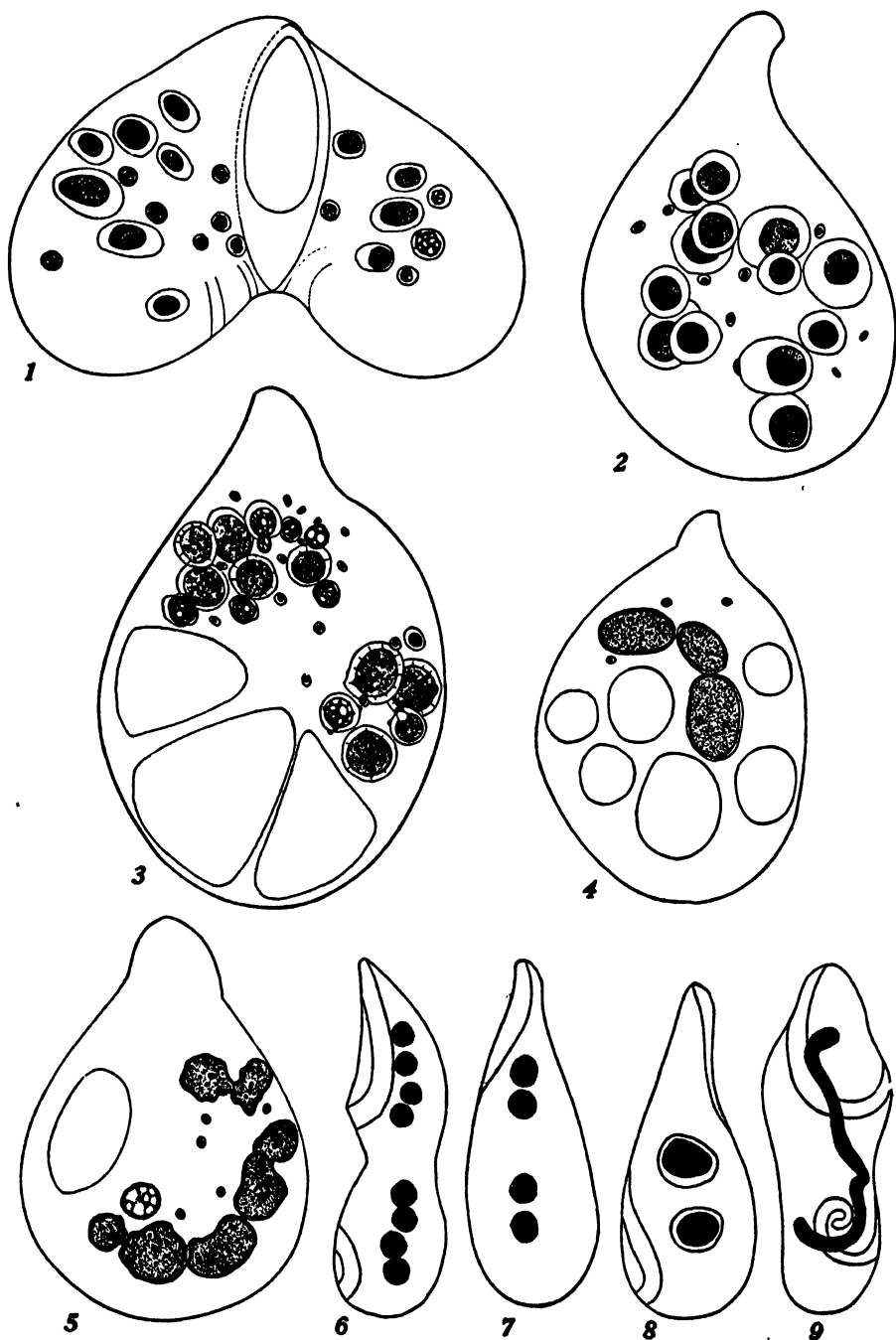


Fig. D. 1-5 drawn $\times 500$; 6-9 $\times 225$.

(Explanation of Fig. D on page 367.)

and intradesmose (pl. 18, fig. 56). As is true in *Uroleptus halseyi* (Calkins, 1930b) and other ciliates, the stainability of the nucleus decreases after the fusion of the pronuclei, and does not begin to increase again until the new macronuclei have begun to develop.

The second and third amphinuclear divisions are similar to the first except that the nuclei are smaller. The chromatin is at first collected into a large mass from which achromatic strands run out to the nuclear membrane (text fig. C, 1, 2; pl. 18, fig. 57). The chromatin then breaks up into granules which collect on the peripheral fibers of a barrel-shaped spindle. The telophases are similar to that of the first amphinuclear division.

REORGANIZATION OF EXCONJUGANTS

Separation.—Separation of the conjugants usually occurs after the third amphinuclear division. The fusion zone disappears at this time, and the endoplasm of the two animals becomes continuous. Vacuoles then appear in the region of union. These merge into a large vacuole (text fig. D, 1), which gradually separates the animals.

Differentiation of new micronuclei and macronuclei.—The products of the third amphinuclear division are structurally alike at the end of the telophase (pl. 18, fig. 58), but almost immediately a noticeable change occurs. In four of these nuclei the chromatin granules condense into a compact mass, and the nuclei gradually decrease in size. These are the new micronuclei. The other four nuclei begin to enlarge, and the chromatin granules to increase in size and spread out toward the periphery. These are the new macronuclei (pl. 18, fig. 59; text fig. C, 3).

Development of the new macronuclei.—The granules of the developing macronuclei rapidly form into what is apparently a spireme. At this stage almost all capacity for nuclear stains is lost (pl. 18, fig. 60). The nuclei increase in size until they reach a diameter of from 10 to 15 μ (pl. 18, figs. 66, 67). The spireme appears to be a continuous thread. It resembles very much a ball of yarn. No loose ends could be detected, as described by Turner (1930) in *Euplotes patella*. The central part is always drawn away from the nuclear membrane, with connecting strands radiating out in all directions. This is

Fig. D. Stages in reorganization of exconjugants. 1 stained with Feulgen, all others with iron haematoxylin. 1-5 drawn $\times 500$; 6-9 $\times 225$.

1. Conjugating pair, showing formation of separation vacuole in the connecting zone.
2. Exconjugant with large basophilic bodies in developing macronuclei. Eight micronuclei visible.
3. Same, showing smaller basophilic bodies, some of which are being extruded from the macronuclei.
4. Late stage in development of macronuclei. Nucleoli beginning to appear.
5. Exconjugant, showing fusion of developing macronuclei.
6. A dividing exconjugant, showing distribution of four developing macronuclei to each daughter individual.
7. Same, showing distribution of two nuclei to each daughter.
8. Early division stage of exconjugant with two developing macronuclei.
9. Typical division figure of young exconjugant after nuclear reorganization has been completed.

probably owing to shrinkage in fixation, but is apparent after the use of any fixative.

There next appears in the spireme a definite split (pl. 19, fig. 68), after which the spireme breaks up into granules which are clearly grouped in pairs (pl. 19, fig. 69). Often the granules are also in fours, as described by Poljansky (1934) for *Bursaria truncatella*. At this time the granules show a great affinity for haematoxylin, and stain faintly with the Feulgen reaction. The paired arrangement of granules does not persist long. Soon they become scattered irregularly throughout the developing nucleus (text figs. C, 4, and D, 1; pl. 19, fig. 70). At this time the affinity for nuclear stains begins to decrease.

The formation of four new macronuclei occurs in only about 46 per cent of exconjugants, as is indicated from study of mass preparations. Any number of nuclei from 1 to 25 have been counted, although from 4 to 10 occur in about 90 per cent of the animals. *Fabrea* shows extreme plasticity of organization comparable to that in many ciliates. Perhaps the most extreme variation, and the one most comparable to *Fabrea*, is described by Diller (1928) in the development of the new macronuclei in *Trichodina*, where the number of nuclei may vary from 1 to 27. In *Trichodina*, as is also true of *Fabrea*, the new macronuclei may be of different sizes, indicating that either division or fusion of nuclei, or possibly both, may have occurred.

The presence of a single developing macronucleus may indicate that a precocious differentiation has occurred after the first amphinuclear division. If after the second division, there would be two. The presence of more than four may indicate additional divisions of one or more of the amphinuclear products before differentiation occurs. In fact, many exconjugants have been found with more than eight undifferentiated amphinuclear products.

However, there is a possibility that amitotic division of developing macronuclei may occur. In many developing nuclei the granules are arranged in the center, with fibrils leading to polar condensations of some kind (pl. 19, fig. 71). In others the division of the granules into two groups seems to be indicated (pl. 19, fig. 72; text fig. C, 4). But it must be emphasized that no further evidence of constriction or division of these nuclei has been observed; so the possibility of amitotic division is to be questioned.

The further development of the young macronuclei to the definitive adult condition requires special mention. Calkins (1930a) states that in *Uroleptus halseyi* chromatin granules showing an affinity for the Feulgen stain gradually replace the matrix of the developing macronuclei, indicating "a gradual accumulation of nucleic acid which is scarce at first in the nucleus but is abundantly developed in later stages."

In *Conchophthirius mytili* and *Ancistruma isseli*, Kidder (1933b; 1933c) describes the formation of chromatin spheres which are cast out of the developing nuclei at each division of the exconjugant in reorganization and distribution of nuclei to daughter individuals.

In *Bursaria truncatella*, Poljansky (1934) believes that the chromatin of the early stages of the macronuclei goes to form the large basophilic "Binnen-

körper." This results in a stage in which only the "Binnenkörper" show any affinity for nuclear stains. Later these basophilic bodies disappear, and the finely dispersed chromatin of the adult macronucleus appears. This is believed by Poljansky to be derived from the breaking down of the "Binnenkörper." Nucleoli begin to form, and the definitive macronuclei are then distributed to daughter individuals through two divisions of the animal.

In *Fabrea* the course of development is very much like that described by Poljansky (1934) in *Bursaria truncatella*. The large granules of the early macronuclei seem to break up into extremely fine ones (pl. 19, fig. 73). At the same time, basophilic bodies of various sizes begin to appear and the rest of the nucleus loses entirely its affinity for nuclear stains. The basophilic bodies stain with all nuclear stains and probably correspond to the "Binnenkörper" of *Bursaria*. They vary in number and size, the larger ones apparently formed by fusion of the smaller. With iron haematoxylin they stain intensely black (text fig. D, 2, 3; pl. 19, figs. 75, 76). The Borrel stain shows them as red bodies. In some nuclei a mass of these red granules may be found at the periphery of the green nucleus, as if they were being extruded (pl. 19, fig. 74).

There is evidence that some of these bodies are thrown out into the cytoplasm of developing macronuclei. Many nuclei show them pushing out toward the periphery of the plastin ground substance (text fig. D, 2, 3). In others they are apparently being cast out through the nuclear membrane. The fact that they decrease in number as development proceeds lends support to this conclusion. The extrusion of chromatin probably represents, as suggested by Diller (1928) and Kidder (1933b), the throwing off of germinal material that is no longer needed in the trophic activity of the adult macronucleus.

After the disappearance of the darkly staining spheres, a rapid increase in chromatin occurs in the developing macronuclei. With the Borrel mixture this appears in the form of dark red granules scattered through the green mass of the nucleus (pl. 19, fig. 77). Later the chromatin granules replace the ground substance entirely, and true nucleoli begin to appear (pl. 19, fig. 78). At this time the nucleoli become quite amoeboid. This may be observed in the living animal as well as in stained preparations. The nuclei are almost invariably lined up in a series, and show pseudopodial outgrowths. In many animals the nuclear membranes are pressed against one another, and in some the actual fusion can be observed (text fig. D, 5).

Experimental evidence also indicates that fusion of developing macronuclei occurs in *Fabrea*. A study was made of 100 conjugating pairs put in drop cultures and the exconjugants isolated immediately after separation. These were observed twice daily, and at each observation some were killed with acetocarmine. In no animal did the division of an exconjugant occur until after the macronucleus had been completely reorganized. This is also reported to be true in *Stentor* (Mulsow, 1913). The process of reorganization required from five to seven days, six being the average. The number of developing macronuclei in those observed varied from four to eight. They increased in size and were still visible as a single row of oval amoeboid bodies in the last

stages before the appearance of a typical band-formed nucleus. The only conclusion that can be drawn is that fusion of nuclei occurred. It is hardly probable that all except one could degenerate so quickly that no traces would be left, or that two divisions of the animal would occur within a few hours, and one daughter die after each division. No evidence of degenerating nuclei or of dead animals was observed.

The objection, advanced by some writers, that the nuclear membranes would interfere with fusion of developing nuclei is not logical, as is clearly demonstrated in ciliates that have more than one macronucleus, because in these the several nuclei always fuse at division into a single contracted body. The fusion of developing macronuclei to form the adult nucleus has been described by Prandtl (1906), Chatton and Perard (1921), Dogiel and Federowa (1925), Jameson (1927), Miyashita (1927), and Dogiel (1928; 1930).

Stolte (1924) states that in *Blepharisma undulans* a U-shaped body appears in one of the developing macronuclei. This is said to burst the surrounding membrane and elongate to form the new macronucleus. This does not agree with the descriptions of *Blepharisma* by Calkins (1912) or Moore (1924), and nothing of this sort occurs in *Fabrea*. U-shaped pieces of the old macronucleus are common (text fig. B, 2), but they disintegrate soon after fertilization.

Many variations in reorganization occur. Out of several hundred exconjugants studied, one was found in which division was occurring, with four macronuclei being distributed to each cell (text fig. D, 6). In one animal there was a dividing exconjugant with two nuclei going to each cell (text fig. D, 7). In several exconjugants there were two typical band-form nuclei. There were some in which two large round nuclei were being distributed to separate daughters (text fig. D, 8), some with one round and one band-form nucleus going to each daughter, and some in which two band-form nuclei were being separated into two individuals by division. This type of distribution by division of the exconjugants is considered the normal method in *Bursaria truncatella* by Poljansky (1934) in contrast to his earlier opinion (Poljansky, 1928) that the nuclei fused. Distribution of this type does occur in *Fabrea*, but such occurrences have been noted in very few individuals in comparison with those in which the nuclei seem to be fusing. And in isolation cultures no exconjugants have divided before nuclear reorganization was complete.

Some exconjugants seem to have no nuclear apparatus of any kind. These are probably degenerate forms in which conjugation did not go to a successful conclusion. They become filled with globules that appear to be fat, and die after swimming around for a few days.

Reconjugation between individuals that have not completed nuclear reorganization has not been observed in *Fabrea*. Triple and quadruple conjugations are quite common, but no cytological study has been made to see if in these all the individuals go through the maturation divisions.

The first division of the exconjugant differs from that of ordinary vegetative individuals in only one respect. The macronucleus contracts in the usual

fashion, but in elongating it pulls out into a longer band than is found in other divisions. This results in a slightly coiled or twisted nucleus during the early elongation of the animal (text fig. D, 9). After the first division, however, the typical form again appears. Nucleoli are less abundant in the macronuclei of animals after conjugation than after a long period of continuous vegetative divisions. This leads to the conclusion that a greater reorganization of substances takes place in the cell during conjugation than occurs in division. As stated before, conjugation is not a death warrant for *Fabrea*, but seems to give rise to a renewal of vitality, as is indicated by rapidly dividing cultures. It is not possible, however, to give any specific information on this point, since no controlled experiments have been carried out with a study of this in mind.

SUMMARY

1. *Fabrea salina* (Henneguy, 1890b) is a heterotrich ciliate of the family Stentoridae. It has been described in the literature under the names *Climacostomum* n. sp. (Stepanow, 1886), *Climacostomum stepanowii* (Entz, 1901), and *Bursalinus synspiralis* (Kalmus, 1929).

2. *Fabrea* has been found only in marshes and lakes of which the salt concentration is greater than that of sea water. In the laboratory it may become adapted to sea water or brackish water, but does not thrive in water of such low concentration.

3. *Fabrea* can be cultured successfully on a medium of concentrated sea water and wheat.

4. The life activities, morphological aspects, encystation, division, and conjugation of *Fabrea* have been described.

5. The external morphology has been found to conform in most details to the descriptions given by Henneguy (1890b) and Fauré-Fremiet (1912).

6. A cilia-free band on the right margin of the adoral zone is considered homologous to a similar band described by Schuberg (1890) and Johnson (1893) as the true peristome in *Stentor coeruleus*.

7. A contractile vacuole, as described by Entz (1901), and an anterior cytoproct, as described by Kalmus (1929), do not occur in the form studied in this laboratory.

8. The cytoplasm has been found to contain both granular and rodlike bodies of chondriosomal nature, some of which may persist after sublimate fixation.

9. The vacuome and Golgi apparatus have been found to be separate inclusions.

10. Fat globules have been demonstrated, but glycogen tests have proved negative.

11. The macronucleus contains both chromatin granules and nucleoli. There is evidence that both chromatin and nucleolar material may be extruded from the nucleus during division and conjugation, and during the development of the new macronucleus after conjugation.

12. The micronuclei vary in number from one to twenty, and cannot be seen

in the living animal. In stained preparations they appear as homogeneous spheres from 1 to 1.5μ in diameter. They may be scattered in the cytoplasm, but are often in close contact with the macronucleus.

13. Encystation seems to be brought on principally by the evaporation of water from the culture dishes.

14. The division rate is highest in cultures with a concentration of about 1.050.

15. Conjugation may be induced by changing the animals from a medium of high salt concentration to a fresh medium of lower concentration.

16. There is no preformed mouth opening or pharynx. Food is guided into the body by long cilia which act as pharyngeal strands leading deep into the endoplasm.

17. A neuromotor system is present. It consists of a motorium, an adoral fibril, and fibrils connecting cilia in the longitudinal rows with the adoral fibril and membranelles.

18. The membranelles of the adoral zone appear to be embedded separately in the cytoplasm. There is evidence that the basal plate described by Henneguy (1890b) is an optical illusion.

19. In binary fission the micronuclei undergo mitotic division. The macronucleus divides by constriction, but undergoes internal reorganization during the process.

20. The old adoral zone is dedifferentiated during the process of division. There is evidence of at least a partial dedifferentiation during conjugation.

21. During conjugation the macronucleus breaks down into spherical bodies which are absorbed into the cytoplasm.

22. The micronuclei in conjugation undergo three maturation divisions. All micronuclei usually take part in the first division, which is of the parachute type.

23. From eight to twelve micronuclei take part in the second maturation division. There is evidence that this is a reduction division, with approximately twelve chromosomes going to each pole of the spindle.

24. From three to six micronuclei complete the third maturation division, but only two nuclei in each individual function as pronuclei.

25. There are typically three amphinuclear divisions, resulting in eight similar nuclei, four of which condense to form micronuclei, and four of which enlarge and develop into the new macronuclei.

26. *Fabrea* exhibits extreme variability in methods of reorganization processes in the exconjugants. In some individuals the developing macronuclei apparently fuse to form the definitive adult nucleus, which assumes its typical band form before the first division of the exconjugant. In other individuals the new macronuclei are distributed by divisions of the exconjugant.

27. Conjugation seems to have a stimulating rather than a depressing effect on individuals in which the process goes to normal completion.

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PLATES

EXPLANATION OF PLATES

All figures drawn with the aid of camera lucida.

Abbreviations for methods of preparation:

B.,	Bouin's fluid
Bo.,	Borrel stain
Ch.,	Champy's fluid
D.,	Delafield's haematoxylin
F.,	Feulgen reaction
Fl.,	Flemming's fluid without acetic acid
H.,	Heidenhain's haematoxylin
K.,	Kull method after Champy
M.,	Mallory's triple stain
M.K.,	Mann-Kopsch osmic impregnation
Os.,	Osmic vapor
S.,	Schaudinn's fluid
S.A.,	Sublimate-acetic in 95 per cent alcohol
Y.,	Yocum's picromercuric fixative
Z.,	Zirkle's copper bichromate fixative

PLATE 15

Fig. 1. Ventral view of *Fabrea salina*, drawn from the living animal. $\times 425$.

Fig. 2. Cyst, drawn from the living animal. $\times 650$.

Fig. 3. Section perpendicular to surface of adoral zone, showing sections of basal lamellae. F. H. $\times 1000$.

Figs. 4-7. Series of sections through the buccal funnel, showing the inner coil of the adoral zone and the fibrils leading into the endoplasm. S. H. $\times 1000$

Fig. 8. Vertical section of adoral zone, parallel to surfaces of basal lamellae. Y. M. $\times 1300$.

Fig. 9. Same. Fl. H. $\times 1300$.

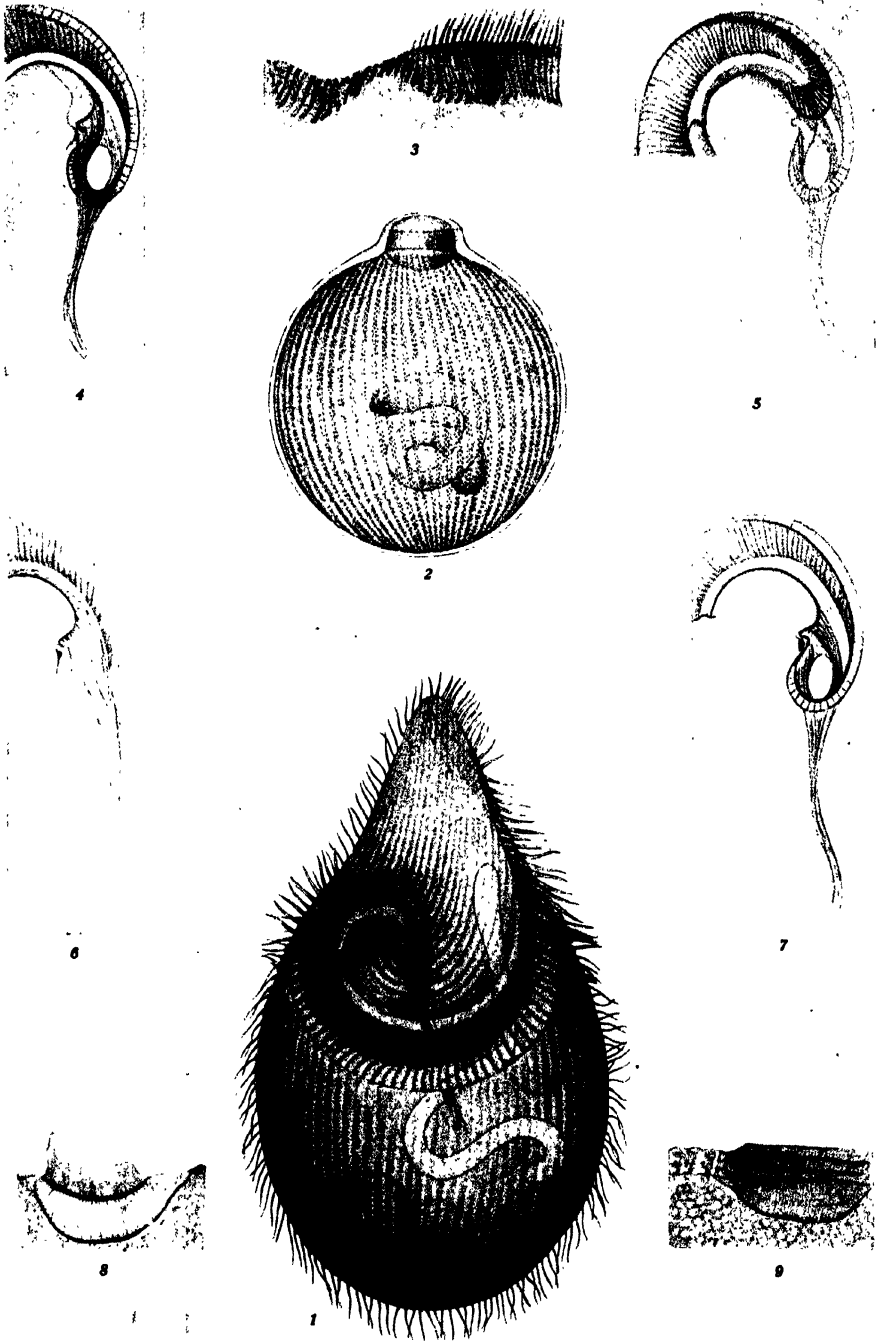


PLATE 16

Fig. 10. *Fabrea salina* at the beginning of division, showing macronucleus with prominent nucleoli. S. H. $\times 420$.

Fig. 11. Early division stage. Micronuclei in metaphase. New adoral zone forming on posterior part of body. S. H. $\times 420$.

Fig. 12. Division stage, showing maximum contraction of the macronucleus. Micronuclei in anaphase. S. H. $\times 420$.

Fig. 13. Same, showing elongation of macronucleus. Micronuclei in telophase. S. F. H. $\times 420$.

Fig. 14. Same, showing early constriction of the body. Micronuclei completely reorganized. S. F. $\times 420$.

Fig. 15. Separation of daughter individuals. Macronuclei connected by a fine strand. S. F. $\times 420$.

Fig. 16. Part of macronucleus, showing finely divided chromatin granules. No nucleoli visible. B. F. $\times 1300$.

Fig. 17. Same. S. F. $\times 1300$.

Fig. 18. Same, showing scattered nucleoli. S. Bo., $\times 1300$.

Fig. 19. Same, showing refractive nucleoli in macronucleus of animal from an old culture. S. F. $\times 1300$.

Fig. 20. Same, showing reticular appearance of chromatin. Fl. H. $\times 1300$.

Fig. 21. Same, showing chromatin as distributed granules. Nucleoli scattered. S. H. $\times 1300$.

Fig. 22. Same, with alveolar appearance. S. A. H. $\times 1300$.

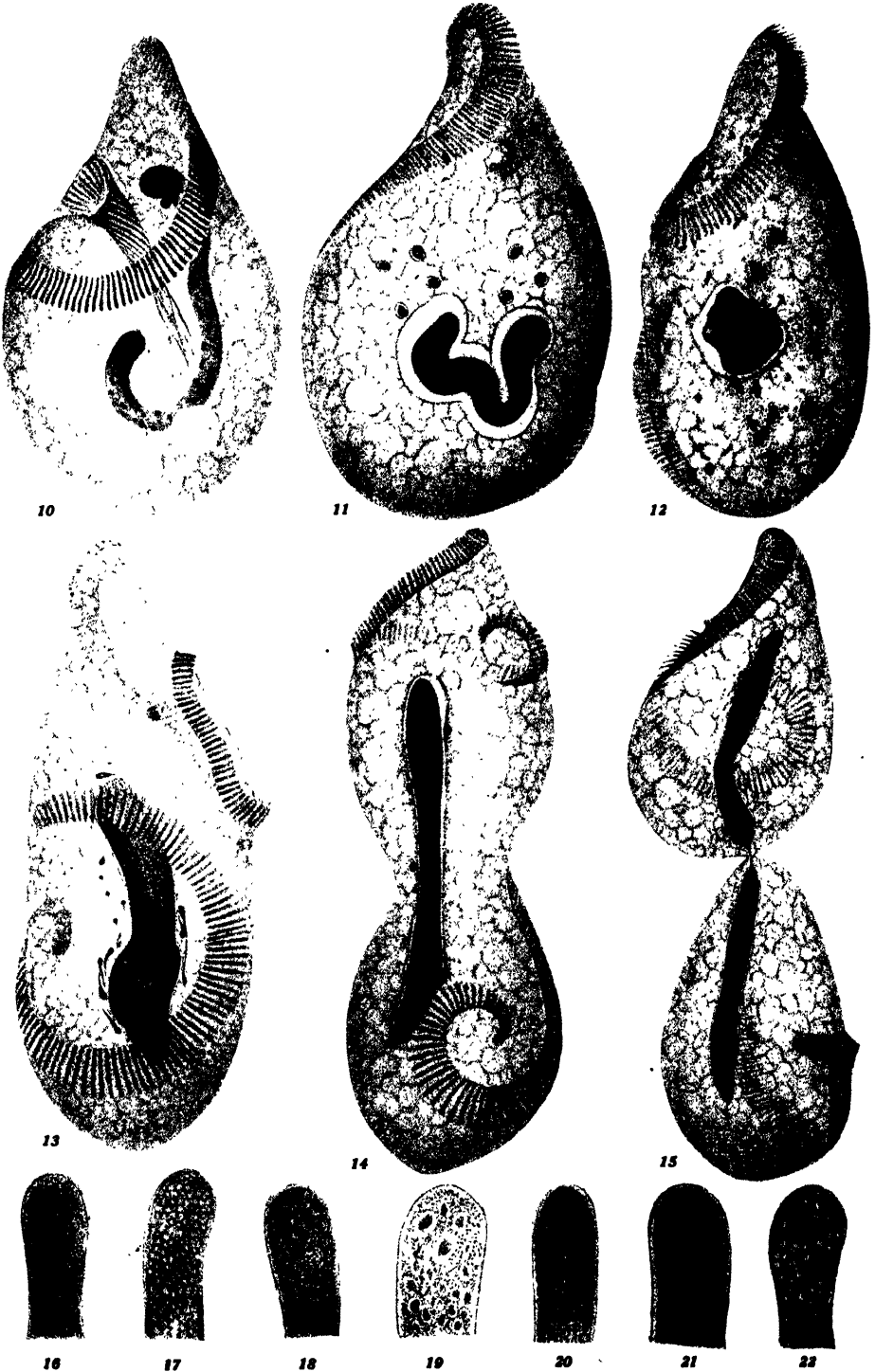


PLATE 17

Fig. 23. Section through adoral zone at the anterior part of the animal, showing mitochondria. Ch. K. $\times 650$.

Fig. 24. Frontal section through buccal funnel, showing motorium and basal lamellae of membranelles. Y. M. $\times 1350$.

Fig. 25. Section through basal lamellae, parallel to surface of adoral zone. Fl. H. $\times 1350$.

Fig. 26. Section of animal in division, showing contraction of macronucleus and formation of new adoral zones. Old adoral zone beginning to dedifferentiate. Fl. H. $\times 440$.

Fig. 27. Section showing Golgi apparatus. Yabroff modification of the Da Fano silver impregnation method. $\times 650$.

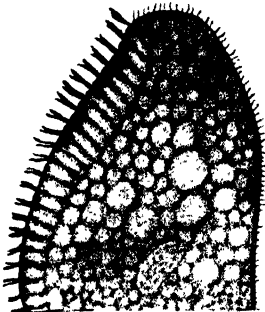
Fig. 28. Transverse section through anterior portion of body, showing Golgi apparatus after osmic impregnation. M. K. $\times 650$.

Fig. 29. Part of body surface in region of the union of adoral zone with frontal field and posterior body stripes. Z. H. $\times 1000$.

Fig. 30. Part of surface area, showing arrangement of stripes and origin of cilia. Os. $\times 1300$.

Fig. 31. Frontal section through adoral zone. Drawn from a combination of two $6\text{-}\mu$ sections. Fl. H. $\times 1000$.

Fig. 32. *Fabrea* killed in Carnoy's fluid and stained with Sudan III, showing fat globules. $\times 450$.



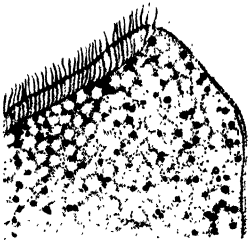
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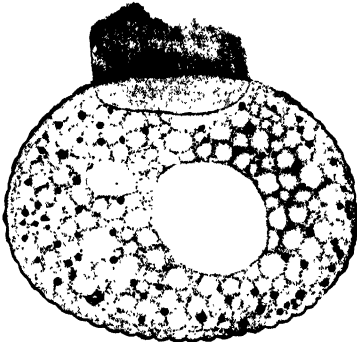
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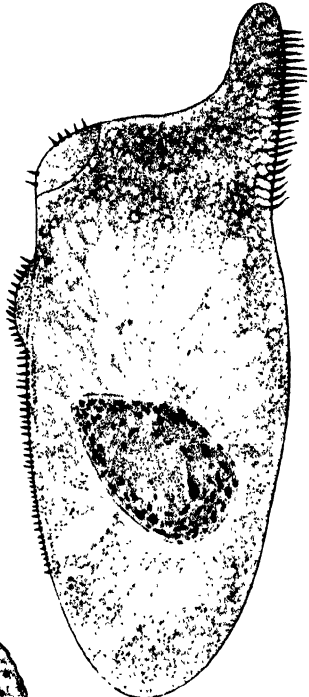
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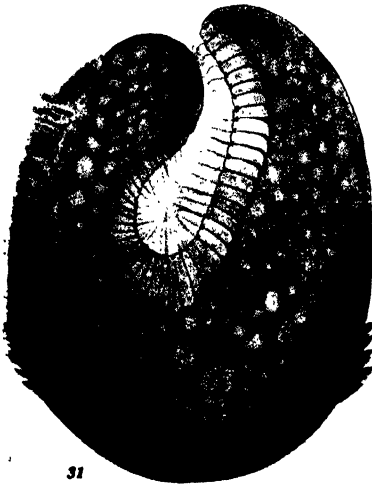
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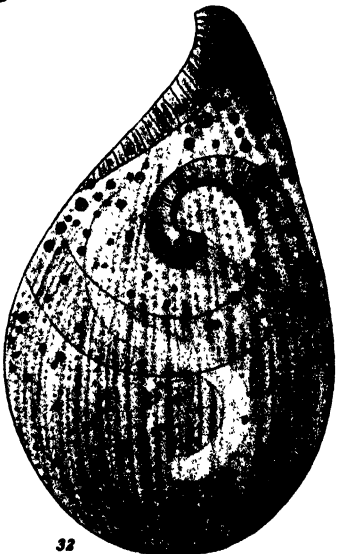
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32

PLATE 18

Figs. 33–40. Micronuclei in division. All $\times 2800$.

Figs. 41–60. Micronuclei in conjugation. All $\times 2800$.

Fig. 33. Resting micronucleus. Fl. H.

Figs. 34–35. Prophase stages of mitosis. S. H.

Fig. 36. Metaphase. S. H.

Fig. 37. Anaphase. S. H.

Figs. 38–40. Telophase stages. S. F. H.

Figs. 41–45. Sequence of prophase stages of the first maturation division. S. H.

Fig. 46. Chromomeres in anaphase of first maturation division, the number too large to be determined. S. H.

Fig. 47. Telophase of first maturation division. S. H.

Fig. 48. Second maturation division, showing approximately 24 chromosomes in center of spindle. S. H.

Fig. 49. Anaphase of second maturation division, showing approximately 12 chromosomes at each pole. S. F.

Fig. 50. Second-maturation telophase. S. H.

Fig. 51. Anaphase of third maturation division, with narrow spindle. S. F.

Fig. 52. Third-maturation telophase. S. H.

Fig. 53. Fusion of pronuclei, showing dense granular zone. S. H.

Figs. 54–55. Polar and equatorial views of synkaryon spindle, showing ragged granular chromosomes. S. H.

Fig. 56. Telophase of first amphinuclear division. S. H.

Fig. 57. Nucleus typical of the two or four amphinuclear stage, showing chromatin collected into a dense mass. S. H.

Fig. 58. Two nuclei after third amphinuclear division. S. H.

Fig. 59. Differentiation of new macronuclei and micronuclei. Macronuclear chromatin swelling, micronuclear chromatin condensing. S. H.

Fig. 60. Later stage in development of nuclei, showing reticulum in new macronucleus. S. H.

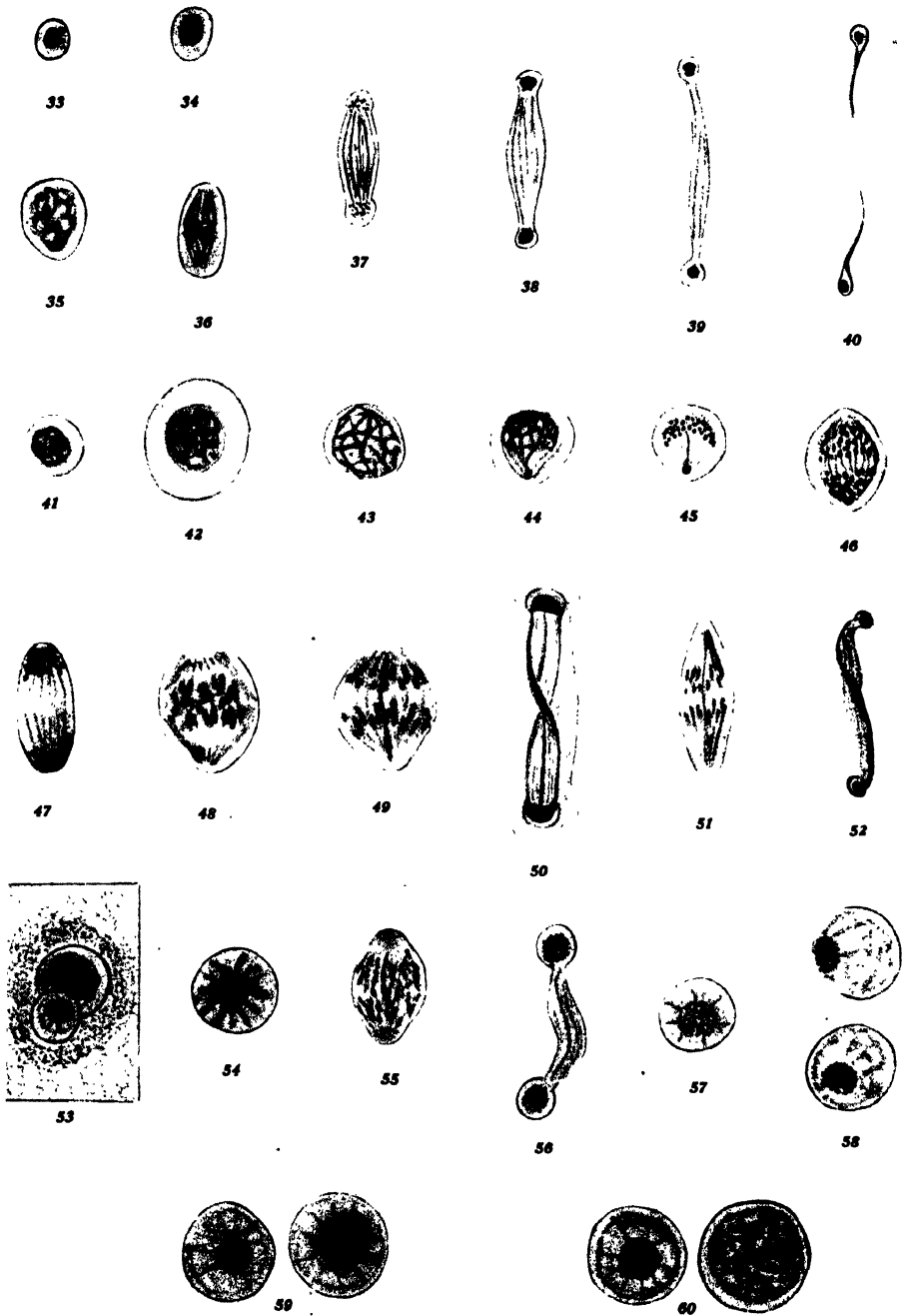


PLATE 19

Figs. 61-65. Degeneration of old macronucleus in conjugation. $\times 1350$.

Figs. 66-79. Development of new macronucleus in exconjugant. $\times 1350$.

Fig. 61. Section of macronuclear fragment at time of second maturation division. S. H.

Figs. 62-65. Stages in degeneration of fragments, showing vacuolization and collection of chromatin into masses. S. F.

Figs. 66-67. Development of spireme in new macronucleus. Nucleus beginning to increase in size. Fl. H.

Fig. 68. Nucleus showing longitudinal split in spireme. S. H.

Fig. 69. Breaking of spireme into pairs of granules. S. H.

Fig. 70. Nucleus after separation of paired granules. S. F.

Figs. 71-72. Pseudo spindle stages often observed. No evidence of constriction of division of nuclei. S. H.

Fig. 73. Breaking down of chromatin granules, with loss of affinity for nuclear stains. S. H.

Fig. 74. Developing macronucleus with basophilic granules at the periphery of the nucleus. S. Bo.

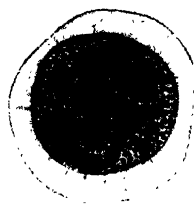
Fig. 75. Section of developing nucleus, showing large basophilic bodies, some of which are vacuolated. Fl. H.

Fig. 76. Nucleus with basophilic body migrating to periphery. S. H.

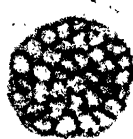
Fig. 77. Nucleus after disappearance of large basophilic bodies. Chromatin granules beginning to appear. S. Bo.

Fig. 78. Later stage in development. Chromatin finely granular. First appearance of nucleoli in exconjugant. S. H.

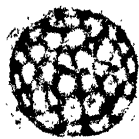
Fig. 79. Fusion of two developing nuclei of animal shown in text fig. D, 5. S. H.



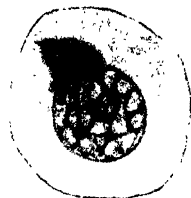
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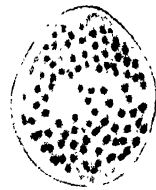
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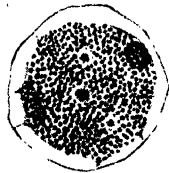
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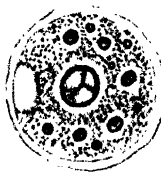
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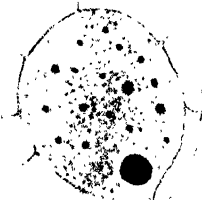
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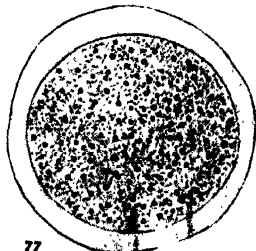
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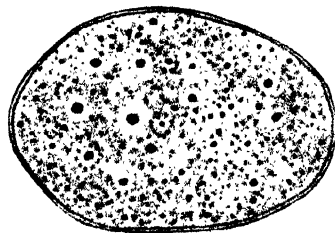
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**CYTOLOGICAL VARIATIONS IN
THE BLOOD AND BLOOD-FORMING
ORGANS OF WHITE-FOOTED MICE
EXPERIMENTALLY INFECTED
WITH TRYPANOSOMA CRUZI**

**BY
SHERWIN F. WOOD**

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INTRODUCTION

INVESTIGATIONS of Ezequiel Dias (1912), Mazza (1926*a, b*), Geoghegan (1929), Niño (1929), and Naegeli (1931) have shown that a lymphocytosis is characteristic of infections with *Trypanosoma cruzi* Chagas, the causative agent of Chagas' disease. The present study was undertaken in an effort to determine what significant changes are produced in the cytology of the blood and spleen of mice experimentally infected with the recently discovered California strain, and whether these agree with the above-mentioned investigations. The writer's findings confirm the conclusion of Kofoed and Donat (1933*a, b, c*) and of Wood (1934*a, b*) that the parasite occurring in California is *Trypanosoma cruzi*.

The author wishes to express appreciation to Dr. C. A. Kofoed, of the Department of Zoölogy, for his interest and valuable suggestions regarding this problem, and to Dr. M. E. Simpson and associates, of the Department of Anatomy, for assistance in the difficult techniques involved, and for criticism of the hematological investigations.

MATERIALS

The recently discovered California strain of *Trypanosoma cruzi* Chagas from laboratory-infected cone-nose bugs (*Triatoma protracta* Uhler) was used for inoculation of animals. Wood (1934*b*), while working out the life cycle of the organism, discovered that certain mammals, namely, several species of white-footed mice (*Peromyscus*), were especially susceptible to the infection. Because of the docility of the Southern Parasitic Mouse (*Peromyscus californicus insignis* Rhoads) and its adaptability to laboratory conditions, the author used this animal for experimentation. Specimens were obtained from Los Angeles and San Diego counties in California, and others were raised in the laboratory from this stock.

METHODS

In the study of the blood and tissues, only adult, sexually mature male mice were used. All animals were weighed before the experiments were begun. The average weight was 30 (25–36) gm. for control mice, and 42 (38–47) gm. for experimental mice. The feces of all mice were examined for intestinal worms. No evidences of worm infection were found, although some animals showed

amoebae and *Trichomonas*. One normal animal was found to be parasitized near the anus by a *Cuterebra* larva after determinations were begun. This probably accounted for a high white-cell count on the fifth day, at which time the larva was removed, with seemingly no ill effects to the mouse. Each mouse was inoculated intraperitoneally with a 0.3 cc. suspension of infective intestinal contents of one adult *Triatoma protracta* in sodium citrate solution (1 gm. of sodium chloride and 1 gm. of sodium citrate in 200 cc. of double-distilled water). No experimentally inoculated mice died from the infection. All control mice were inoculated intraperitoneally with 0.3 cc. of sodium citrate solution.

Because of the reported stimulation of erythropoiesis in mice by repeated bleeding (Scarborough, 1930), sufficient time, namely, six days, was allowed between samplings to avoid interference with the normal process. The total period of sampling was thirty-six days. In order to cover every day of the infection, counts were made on two mice (one infected and one control) on the 1st, 7th, 13th, 19th, 25th, and 31st day after inoculation, on two others on the 2d, 8th, 14th, 20th, 26th, and 32d day, and so forth, until six sets were completed. In this manner the effects of experimental hemorrhage were avoided, and the site of sampling had time to heal before the next samples were taken. Blood was taken from the veins of the ears, which are large and almost hairless in these mice, the vessel being cut transversely with the sharp corner of a razor blade.

Red- and white-cell counts were made according to common practice (Stitt, 1927) with a certified Bausch and Lomb hemocytometer. Hemoglobin estimations were made with a Hellige normal hemometer. All readings were taken after one-half hour to insure complete change of the hemoglobin to acid hematin. Reticulocyte counts were made according to Pappenheim's (1907) method. The percentage of reticulocytes for one thousand red cells was noted. Differential leucocyte counts were made upon smears stained with May-Giemsa. For nearly every animal two hundred white blood cells per smear were counted in such a way that all four sides of the smear were sampled. The average number is here given, followed in parentheses by the range for each cell type.

The procedure for making the determinations was as follows. The animal was placed in a cylinder of $\frac{5}{16}$ -inch square galvanized wire-cloth netting (20 gauge), one end of which was pointed and soldered together. The mouse was held in place by inserting two small sticks behind it through the netting. This arrangement allowed the animal to be in a normal resting position, with the ears protruding through the netting. The *right* ear was cleaned with 95 per cent alcohol and then gently wiped dry with a piece of sterile cotton. One or two veins, depending upon the rapidity of bleeding, were slit transversely with a razor and the blood taken into the hemometer pipette and immediately diluted. Then, one or two drops of blood from the ear were diluted with Ringer's solution for the reticulocyte count. The *left* ear was cleaned and wiped and one vein slit and the sample taken in the red pipette to the 0.1 mark only. The dilution was made with Hayem's solution. The cut was then wiped clean and a sample taken in the white pipette to the 0.1 mark and diluted with 1 per cent

acetic acid in distilled water. The cut was again wiped clean and the next one or two drops were used for smears.

Smears were tested for oxydase ferment by the following method. Air-dried films were treated with a benzidine solution (100 mg. benzidine, 20 cc. methyl alcohol, 5 cc. distilled water, 1 drop of hydrogen peroxide) which after 30 seconds was diluted one-half with a 0.1 per cent aqueous copper-sulphate solution, and this allowed to act for 2 minutes. The slides were washed in distilled water and then stained with Giemsa's blood stain for 10 minutes, washed in distilled water, and dried. After being thoroughly dried, they were mounted in Grüber's euparal-green or neutral Canada balsam. Smears fixed by Knoll's (1932) method and treated with benzidine solution alone gave the best results.

Normal blood was studied with neutral red and Janus green in separate solutions as well as mixtures. Only fresh preparations were used, the preparations being immediately discarded on evidence of degeneration. Smears of the blood and impression smears of the spleen and of the bone marrow stained with May-Giemsa, Jenner-Giemsa, and Pappenheim's panchrome were also studied. Sections of the spleen, sternum, and lymph nodes were prepared after infiltration and embedding in mixtures of nitrocellulose and ether-alcohol by the method of Dr. Alexis Koneff, of the Department of Anatomy, University of California. All sections of the spleen were sagittal or parasagittal, and were mounted on slides and the embedding mixture removed after Maximow's (1909) method. Some sections of all tissues were stained in Delafield's hematoxylin and eosin. Spleen sections were stained with Pappenheim's (1911) panchrome, Mallory-azan, silver-gold-Harris's hematoxylin-Van Giesen, Harris's hematoxylin and Van Giesen, and May-Grünwald-Giemsa (Petri, 1930). Sections of sternum were stained with the three combinations last mentioned.

MORPHOLOGY OF THE NORMAL BLOOD

The *red blood corpuscles* (pl. 20, fig. 2) are typically small biconcave disks. The average number per cubic millimeter for twelve mice was 10,600,000 (9,600,000–12,910,000). The average diameter of one hundred corpuscles chosen at random from air-dried smears was 5.3μ , with a range of 4.2 – 6.9μ . Throughout the blood of normal animals Howell-Jolly bodies were seen occasionally in the red corpuscles, especially while differential leucocyte counts were being made, but the actual rate per thousand red corpuscles was very small, since only one or two were found on a few slides. The average percentage of reticulocytes for twelve mice was 2.5, and of polychromatic corpuscles 1.0.

The differentiation of leucocyte types follows the work of Petri (1933, 1934) for the blood of white mice. The terminology of Pappenheim and Hirschfeld (1920) has been used in descriptions of cell structure. In contrast to the blood of white mice, the nuclei of all granular leucocytes in the blood of *Peromyscus* are typically ring-shaped (pl. 20, figs. 1, 3–12). This ringlike appearance is often masked by the overlapping of adjacent parts, so that at times, with deeply stained cells, a typical horseshoe-shaped nucleus may appear. Close inspection, however, reveals their ring character. The width of the ring in the neutrophils is such that it allows for some flexibility and twisting, in con-

trast to the nuclei of eosinophils and basophils. In a few cells, a nucleus was found with no connecting threads between the lobes. However, it is doubtful whether cells with this type of nucleus are normal constituents of the blood; they may be artifacts caused by smearing or fixation.

The average number of leucocytes per cubic millimeter for twelve mice was 10,100 (3,750–16,750). Noticeable in the differential leucocyte counts of normal mice is the large variation in numbers of type *a* neutrophils, type *a* eosinophils, and medium and small lymphocytes.

The *neutrophil leucocytes* are divided into three types: multilobulated, segmented, and unsegmented. The multilobulated form (pl. 20, fig. 5) is rare, constituting 0.29 (0.0–1.0) per cent of the white cells. The nucleus of this type is distinctly pinched off into three to five definite lobules connected by narrow chromatin strands. The contour of the lobules is smooth. The segmented type (pl. 20, figs. 6, 7) is most numerous. There are two rather distinct stages of the segmented type, which are designated as *a* and *b*. Type *a* (pl. 20, fig. 6) is applied to a nucleus which is distinctly segmented in several places, in contrast to type *b* (pl. 20, fig. 7), which shows only one distinct segmentation. Segmentation refers to the constriction of distinct masses of the nucleus from one another, leaving only narrow connections. This differs from the lobulated condition only in the width of the connecting part between separate segments. Here it is usually a definite band, in contrast to the threadlike connections between the lobules of the multilobulated type. Thus, type *a* refers to a segmented and lobulated arrangement of the nuclear mass, whereas type *b* refers to a single segmentation without distinct lobulation. The contour of the nucleus of type *a* is irregular and uneven, whereas that of type *b* tends to be smoother. Type *a* constitutes 26.16 (12.5–46.5) per cent, whereas type *b* represents only 0.70 (0.0–1.5) per cent. The unsegmented type (pl. 20, fig. 8) has a simple, round, even-contoured, ringlike nucleus with a definite aperture. It is rarely found in the circulating blood, and then not in all animals.

The nucleus of all neutrophils is fundamentally the same in structure. The chromatin (basichromatin) is distinctly clumped or banded, separated within the lobes and between the lobes by narrow parachromatin (oxychromatin) spaces. Sometimes the clumps are broken up, but the chromatic parts retain a homogeneous deep-staining capacity. The chromatin masses of the multilobular type (pl. 20, fig. 5) tend to be darker, more homogeneous, and more compact, suggestive of older cells and the beginning of nuclear degeneration. The nuclei of the segmented types (pl. 20, figs. 6, 7) show less dense chromatin masses separated by distinct spaces. The unsegmented type (pl. 20, fig. 8) shows the youngest type of nucleus, for the chromatin appears in definite narrow strands separated by narrow parachromatin spaces.

The cytoplasm of the various types of neutrophils varies less than does their nuclear structure. In the older cells, with multilobulated and segmented nuclei, the spongioplasmatic network seems to have disappeared, leaving a distinctly oxyphilic paraplast. However, this is not always so, since sometimes the cytoplasm has a basophilic appearance. In the unsegmented type, the blue-staining spongioplasm tends to overcast the pink-staining paraplast, so that

the cytoplasm has a definite blue, netted appearance. The average diameter of thirty segmented type *a* neutrophils was 10.8μ , with cells ranging in size from 8.4 to 11.7μ .

The neutrophil granules are less distinct when destaining is prolonged to bring out differentiation of lymphocytes. The granules are small, irregular in size and shape, and are purple-violet to light pink (pseudo eosinophilic). They are best seen in overstained parts of the smear, where they stand out as separate, distinct particles. Not all the granules stain with the same intensity, for some show a definite pinkish cast even in the more heavily stained cells. Most of the cells, except the unsegmented type, have many granules scattered throughout the cytoplasm.

The *eosinophil leucocytes* (pl. 20, figs. 9, 10) are of two types, on the basis of nuclear structure. Here, in contrast to the neutrophils, the nuclei of both types are smooth-contoured, but the nucleus of type *a* (pl. 20, fig. 9) is more regular in shape than the nucleus of type *b* (pl. 20, fig. 10). The width of the chromatin mass in type *a* is greater than that of the segmented neutrophils and, when in the form of a circle, surrounds a large aperture. The nucleus of type *a* may be circular, knotted at one end in a figure 8, or folded upon itself in the shape of a horseshoe. The nucleus of type *b* is regular in outline, not twisted, but in simple circular form, enclosing a much smaller aperture. Type *a* constitutes 6.62 (1.5–17.0) per cent of the white cells, whereas type *b* constitutes 0.37 (0.0–2.0) per cent.

The nucleus of eosinophils of type *a* (pl. 20, fig. 9) shows fine and coarse strands or clumps of chromatin scattered throughout the nucleus and separated by narrow parachromatin spaces. The chromatin of type *b* (pl. 20, fig. 10) is more homogeneous in appearance, being distributed throughout the nucleus in broader bands and separated by parachromatin spaces of varying widths.

The cytoplasm of the eosinophils is basophilic where visible among the granules. There is no evidence of a netlike meshwork of spongioplasm. The average diameter of thirty type *a* eosinophils was 10.6μ , with cells ranging in size from 9.3 to 11.7μ .

The eosinophil granules are large, round, highly refractile bodies of uniform size and shape scattered throughout the cytoplasm and in most cells they fill it completely. They vary in color from a distinct dark brownish orange to orange-pink.

The *basophil leucocytes* (pl. 20, figs. 11, 12) of the circulating blood represent two types on the basis of nuclear structure. The nuclei of both are regular in outline, like those of the eosinophils. The nucleus of type *a* (pl. 20, fig. 11) appears either in circular form or in the shape of a figure 8, but is not as plastic as that of the eosinophils or neutrophils, possibly because of its greater width. The nucleus of type *b* (pl. 20, fig. 12) has a much smaller aperture and is always of simple circular form. Sometimes clumps of granules obscure the hole in the nucleus. Type *a* is represented by 2.75 (0.5–11.5) per cent, whereas type *b* constitutes 0.50 (0.0–2.0) per cent.

The nucleus of basophils shows broad, homogeneous chromatin masses sep-

arated by narrow parachromatin spaces. Some of the older cells of type *a* show more parachromatin, tending to break up the homogeneous aspect of the nucleus.

The cytoplasm of the basophil leucocyte is bluish (basophilic) where visible, although in some cells there may be a pinkish cast. The average diameter of thirty type *a* basophils was 11.6μ , with cells ranging in size from 9.6 to 13.8μ .

The basophil granules are large, uniform-sized, dark-purple-staining, ovoidal bodies which are closely crowded together and generally fill the cytoplasm completely.

The *small lymphocytes* (pl. 20, fig. 17) have a simple round, or slightly indented, eccentric nucleus which stains very deeply because of the broad, homogeneous, interconnecting chromatin masses. The parachromatin spaces are limited to narrow areas between the chromatin masses. They constitute 43.66 (18.0–61.0) per cent of the total white cells.

The cytoplasm of the small lymphocytes is limited to a very narrow zone completely encircling the nucleus. Generally, the cytoplasm is visible on one side only, and is deeply basophilic, with a distinct spongioplasmatic network. The tint varies from dark to light blue, and the outermost rim is usually darkest, with a lighter area immediately surrounding the nucleus or at its indentation. Where the cytoplasm is limited to one side of the cell, the characteristic perinuclear halo is also visible only on that side. The paraplasm is blue to gray-blue. The average diameter of thirty small lymphocytes was 7.8μ .

The *medium-sized lymphocytes* (pl. 20, fig. 19) often show the typical nuclear structure of small lymphocytes. Sometimes the nucleus of these cells shows more definite chromatin clumps or masses separated by greater parachromatin spaces. Medium-sized lymphocytes constitute 9.41 (3.5–23.5) per cent of the total white cells.

The cytoplasm of the medium-sized lymphocytes is a broader zone which encircles the nucleus completely. Occasionally the nucleus is decidedly eccentric, but usually some cytoplasm is visible all around it. The cytoplasm of these lymphocytes stains lighter blue than that of the small lymphocyte, tending to show a broader perinuclear halo. The average diameter of thirty medium-sized lymphocytes was 9.3μ .

The azure granules of the small and medium-sized lymphocytes are variable in size and scattered throughout the cytoplasm. They stain pale violet to pink. The larger cells usually show more granules than the smaller cells. In some cells there appear definite groups of granules besides other large homogeneous granules (pl. 20, fig. 18). The appearance of these bodies suggests either ingested platelets, or extranuclear chromatin masses, or Kurloff bodies, but their exact nature is unknown. These bodies were found but sparsely in the lymphocytes and mononuclears of the blood of both normal and infected mice.

The *large lymphocytes* (pl. 20, fig. 16) show noticeable structural variation. Some have simple, indented, or kidney-shaped nuclei which show very distinct broad chromatin clumps and masses. The parachromatin spaces are narrow or slightly broader areas between the chromatin masses. These large lymphocytes are easily recognized because of the close similarity of their nu-

clear structure to that of the small and medium-sized lymphocytes. Rarely, cells with a hole in the nucleus were found (myelocytes ?). There are other large lymphocytes in which the chromatin is separated into more or less distinct clumps or masses with wider parachromatin spaces. In some cells there are only one or two distinct homogeneous chromatin masses in the middle or at one side of the nucleus, the rest of the nucleus showing a definite meshwork of narrow chromatin strands and narrow parachromatin spaces. These cells seem to be borderline examples between the large lymphocytes and the mononuclears and on the basis of nuclear structure alone are difficult to distinguish. The large lymphocytes make up 5.08 (2.5–9.0) per cent of the total white cells.

The cytoplasm of the large lymphocytes shows a broad protoplasmic zone exhibiting various degrees of basophilia. A definite perinuclear halo is usually visible. The dark blue spongioplasm is most distinct toward the periphery of the cell, whereas the bluish gray paraplast is most distinct next to the nucleus or in the region of the nuclear indentation. In some cells, however, the cytoplasm is limited to a narrow zone around a very large nucleus. The presence of a distinct perinuclear halo within a broad, light-staining cytoplasm, plus the characteristic nuclear features, makes accurate classification possible. The average diameter of thirty large lymphocytes was 10.8μ . The range in size of all forms of lymphocytes was from 6.3 to 13.8μ .

The azure granules of the large lymphocytes are few to many and are irregularly scattered throughout the cytoplasm. In some cells there are large azurophilic granules about one-half the size of basophil granules.

The *mononuclear cells* (pl. 20, figs. 13, 14) are among the largest leucocytes of the blood. They constitute 4.66 (1.0–10.5) per cent of the white cells. The largest cell measured 13.8μ , the same as the largest lymphocyte. The nucleus is simple, indented, kidney-shaped, horseshoe-shaped, or sometimes coarsely lobular. The chromatin is distributed throughout the nucleus in fine strands and clumps which are separated by equally extensive areas of parachromatin. This results in a netted or meshlike homogeneous appearance of the nucleus, in contrast to the clumped chromatin masses seen in the nuclei of lymphocytes. In some mononuclears there may be one or two more extensive chromatin masses, with the rest of the nucleus showing the typical meshwork of chromatin and parachromatin. The mononuclears lack the distinct halo and structural uniformity of the cytoplasm of the large lymphocytes. In some cells the edges of the nucleus tend to be composed of an interrupted narrow band of homogeneous-appearing chromatin strips.

The cytoplasm of the mononuclears shows a distinct, darkly stained, bluish, spongioplasmatic network with a lighter-staining, often pinkish or gray, paraplast. The netted and vacuolated aspect gives as distinctive an appearance to the cytoplasm as does the meshwork of chromatin and parachromatin to the nucleus. Most mononuclears show no evidence of a lighter area immediately surrounding the nucleus. Some show a perinuclear halo much like that of the lymphocytes. Sometimes there are several large vacuoles in the cytoplasm. The average diameter of thirty mononuclears was 11.7μ , ranging from 10.2 to 13.8μ .

The azure granules of the mononuclears are numerous, faintly visible, and

irregularly scattered throughout the cytoplasm. Sometimes there are a few larger azurophilic granules here, as in the large lymphocytes.

The *blood platelets* (pl. 20, fig. 2) are small, usually rounded, masses of protoplasm containing a group of small chromatin-like granules. These distinctly outlined basophilic, protoplasmic masses are irregular in size, shape, and number of the chromatin-like granules within the protoplasm, and range in diameter from 1 to 3μ .

The oxydase reaction was positive in all types of neutrophil and eosinophil leucocytes (pl. 20, figs. 1, 3, 4), and negative in all types of basophil leucocytes, lymphocytes, and mononuclears. The granules of the neutrophil leucocytes after fixation by Knoll's (1932) method gave a pronounced positive reaction (pl. 20, fig. 1). The specific granules appear as irregularly shaped, yellowish green masses which tend to be spherical in form. The cytoplasm of some cells is almost completely filled with these granules. Other cells of the same type may show few or many granules, but all types of cells show at least a few granules in their cytoplasm. There seemed to be fewer oxydase granules per cell in slides treated without the fixative. Some granules seemed to give a stronger reaction than others, so that in some cells all grades of color intensity were observed. With copper sulphate the granules are greenish blue after Knoll's fixative, but without the fixative they have a distinct bluish tint. Knoll found that the oxydase reaction in neutrophils of rodents (*Mus musculus* and others) was exceedingly variable with respect to shape and number of granules. Most rodents showed the reaction, but the guinea pig and rabbit showed oxydase-negative neutrophils. The reaction was always positive in eosinophils and negative for basophils and lymphocytes. Most of the monocytes from the different species of animals gave no reaction at all, whereas in some forms, such as the monotremes, there was a positive reaction, although even there it was by no means so definite as that observed in the eosinophils and neutrophils.

The oxydase reaction of eosinophil leucocytes (pl. 20, fig. 3) was more decidedly positive than that of the neutrophils. Whether or not a fixative was used, the eosinophils always exhibited distinct, rounded, highly refractile, yellowish brown to greenish granules which usually filled the cytoplasm completely. With copper sulphate these granules did not react, thus tending to retain the tint from the benzidine treatment only.

Supravital staining differentiates the leucocytes chiefly by the appearance of the specific granules, but also by the distribution of the mitochondria and the appearance of the segregation apparatus. The neutrophils show distinct, small, nonrefractile granules. The eosinophil granules are large and refractile, and the basophil granules are largest of all and nonrefractile. The lymphocytes show appreciable diversity of structure and type. The forms seen here lend support to the finding in stained smears of many different types of lymphocytes. The mononuclears are differentiated from the others by the large, often indented nucleus, the distinct group of reddish brown granules at the indentation of the nucleus, and the numerous mitochondrial granules and rodlets scattered throughout the cytoplasm or more uniformly grouped around the mass of neutral red-staining granules.

MORPHOLOGY OF BLOOD INFECTED WITH
TRYPANOSOMA CRUZI

Recorded data show no differences between normal and infected animals with respect to the number of red and of white corpuscles, hemoglobin, reticulocytes, and polychromasia. There were some changes in the differential leucocyte counts, as is noted below. From the incomplete survey of the normal blood no final comparison can be made of the size and shape of the red corpuscles in different animals, and in the same animal at different times. However, one animal which had five times the usual dosage of infective material, having been inoculated daily for five consecutive days from a total of five bugs, showed increased polychromasia. There were 4.0 per cent of these cells on the 17th day following the first inoculation, and 4.7 per cent on the 20th day, in contrast to 0.5 per cent and 0.7 per cent, respectively, for the controls, and 1.2 per cent and 1.6 per cent, respectively, for the experimental mice with a single inoculation.

The increase in the number of large lymphocytes (pl. 20, fig. 15) in later stages of the infection was the most constant and striking feature in the microscopic picture of the circulating leucocytes. The blood of experimental mice showed an increase of 5.71 (0.0–15.5) per cent per day from the 14th to the 36th day (except the 25th and 31st days, each minus 1.5 per cent) of the infection. Although this indicates only a small change, the difference is readily noted in counting. The large lymphocytes, as a whole, are more uniform not only in size but also in structure, those from infected animals having well-defined, broad interconnecting chromatin bands with narrow parachromatin spaces. The zone of cytoplasm is broad and lightly basophilic, with large intracytoplasmic granules in some cells. The irregularity of the nucleus in some of these cells and its dense homogeneous appearance suggest the beginning of karyolysis, and some of these larger granules may be a product of this action. Besides these more numerous, typical, large lymphocytes there appear other lymphocytes which show more widely separated, definite chromatin clumps characteristic of younger cells of the lymphocytic series.

The eosinophils (type *a*) showed an average numerical decrease of 5.81 (0.5–14.67) per cent per day from the 26th to 36th day after inoculation, except the 34th day (+2.5 per cent). This is substantiated by the increased number of these cells in the spleen. Evidently, their response is enlisted at the sites of activity in resistance to the infection.

The neutrophils showed a tendency toward greater irregularity in structure and lobulation of the nucleus in some infected animals. Most cells of normal blood showed three to five lobules, whereas many in infected animals showed six to eight.

The platelets probably decreased in number during the first part of the infection, since the ears of infected mice bled more freely, suggesting interference with the clotting mechanism. No counts were made, but the appearance of more megakaryocytes in the spleen and bone marrow suggests that such an effect was present.

MORPHOLOGY OF THE NORMAL SPLEEN

The normal spleens were taken from three mice the weights of which were 28, 34 (pl. 21, figs. 20, 23; pl. 22, figs. 24, 26), and 47 gm., respectively. The histology of the normal spleen of the Southern Parasitic Mouse is much like that of man, except that in the mouse the follicles of the white pulp are larger and more extensive in relation to the size of the spleen. Its structure is similar to that of the spleen in the white mouse (Hoyer, 1894; Downey and Weidenreich, 1912; Jaffé, 1921, 1931; Jolly, 1923; Klienenberger and Carl, 1927; Hartmann, 1930; Maximow and Bloom, 1931), the rabbit (Hoyer, 1894; Dominici, 1900a, 1901; Jaffé, 1931), and the rat (Hu, 1931; Jaffé, 1931). In comparison with the extent of the spleen in parasagittal section, the follicles in *Peromyscus*, which generally stand out sharply from the surrounding red pulp, are approximately one-fourth the width of the organ, rarely larger. Since the masses of lymphocytes which constitute the follicles are distributed along the arterial channels, their course through the spleen is variable.

Most follicles show definite secondary nodules or the beginnings of such nodules (pl. 21, fig. 20; pl. 22, fig. 24). These secondary nodules consist of medium and large lymphocytes and intermediate forms with lymphoblasts scattered throughout, as well as phagocytes and fixed reticular cells. However, these germinal centers are always enclosed within masses of smaller lymphocytes of the primary nodules, even though there are only a few cell layers between the edge of the secondary nodule and the reticular membrane inside the marginal zone (Downey and Weidenreich, 1912). Sometimes the follicles show only the dense masses of small lymphocytes with scattered larger lymphocytes, lymphoblasts, and reticular cells characteristic of the primary nodules. Outside the reticular membrane around the follicle is the narrow marginal zone composed mostly of medium-sized and large lymphocytes and infiltrated with red blood corpuscles, small lymphocytes, and large and medium-sized lymphoblasts from the red pulp. In some places the marginal zone is very narrow and the venous sinuses of the red pulp lie close to the reticular membrane of the follicle (Hartmann, 1930). However, no venous sinuses were found in the marginal zone.

The red pulp constitutes relatively wide areas or zones between the follicles (pl. 21, fig. 20). The spaces between the sinuses in the red pulp are heavily infiltrated with lymphocytes, lymphoblasts, eosinophil myelocytes, eosinophil and neutrophil leucocytes, free reticular cells (macrophages), erythroblasts, a few plasma cells, and a few megakaryocytes. The nuclei of the megakaryocytes appear either as mononuclear or multilobulated, the latter form being the predominant type. The general appearance of the red pulp, however, is that of a loose meshwork with scattered free cells in and among the venous sinuses, which are well filled with red corpuscles, and here and there small groups of polychromatophylic erythroblasts (pl. 21, fig. 23). The writer's findings on the free cellular constituents of the red pulp of the spleen of *Peromyscus* agree in general with the findings for the spleen of the white mouse by Downey and Weidenreich (1912), Jaffé (1921, 1931), Jolly (1923),

Klienberger and Carl (1927), Hartmann (1930), and Maximow and Bloom (1931). Jolly (1923) says the megakaryocytes are numerous, whereas Maximow and Bloom (1931) say they occur in small numbers. Klienberger and Carl (1927) found them sometimes numerous, sometimes scanty. Jaffé (1931) found that the distribution of all pulp cells was very irregular, whereas Hartmann (1930) found that megakaryocytes accumulate in the regions of the capsule and trabeculae. The three normal spleens of *Peromyscus* all showed megakaryocytes widely scattered in small numbers throughout the organ but tending to accumulate near the capsule and trabeculae. Hartmann (1930) states that mast cells are generally found in the spleen of white mice, but these were not noted inside the spleen of *Peromyscus*. Only small amounts of pigment were found here in the pulp, in agreement with the findings of Hartmann (1930) for white mice.

The distribution of the connective tissue and reticular framework of the organ is similar to that of the human spleen. The capsule and trabeculae have few smooth muscle cells as in the spleen of white mice (Hoyer, 1894; Hartmann, 1930). The reticulum of the spleen of *Peromyscus* is sharply defined after silver-gold treatment as distinct, coarse, interconnecting, rounded fibrils, which is in agreement with the findings of Jolly (1923) for white mice. Hoyer (1894) reported the presence of a fine, delicate reticulum in the spleen of white mice, but admitted difficulties with technique. The size of the spleen and degree of development of the follicles vary with the age of the animal. The older (heavier) mice show more secondary nodules in the follicles, whereas the younger mice show fewer secondary and mostly primary nodules.

CHANGES IN THE TISSUES WITH INFECTION

SPLEEN

Five infected mice weighing 20, 33, 41, 43, and 50 gm., respectively, were used in studies of the changes caused in the spleen by infection. No differences were noted in the amount or distribution of connective tissue or reticulum in normal and infected animals. The general reaction shown in varying degrees by all infected spleens was toward increased cellular proliferation (pl. 21, figs. 21, 22; pl. 22, fig. 25), especially in the germinal centers of the follicles and to some degree in the free cells of the red pulp. Some spleens were enlarged as much as twice the normal, whereas others showed no increase in size. Enlargement of the spleen is a common accompaniment of infection with *Trypanosoma cruzi*, as pointed out by Castellani and Chalmers (1919), Crowell (1923), Mazza (1926b), Niño (1929), and DeCoursey (1935).

The follicles tended definitely to increase in size, becoming three to five times larger than normal. The entire aspect of the follicle was changed, owing to the hyperplasia of the cells of the secondary nodules, as evidenced by increased numbers of division figures in all stages of mitosis and the partial to nearly complete obliteration of the compact small lymphocyte masses of the primary nodules (pl. 22, fig. 25). The cells of the secondary nodules are chiefly large and medium-sized lymphocytes and lymphoblasts, with fixed and

free reticular cells (macrophages) scattered throughout. There was a marked increase in the number of free reticular cells the protoplasm of which was crowded with ingested cellular remains in various stages of degeneration. In extreme cases the character of the follicle was entirely changed, owing to the obliteration of the primary nodules by the hyperplasia of the cells in the secondary nodules. Mitotic figures were then common throughout the follicles. More often, at one side or another, or almost completely enclosing the secondary nodule, was a narrow zone of small lymphocytes of the primary nodule, just inside the reticular membrane of the follicle. The marginal zone tended to broaden in some instances and become more completely infiltrated with cells of the red pulp, though retaining its homogeneous-appearing zonation surrounding the follicle, a result of the preponderance of medium-sized and large lymphocytes.

The red pulp, in extreme examples, showed under low magnification a decidedly blotchy appearance (pl. 21, fig. 21), owing to the presence of compact clumps of polychromatophylic erythroblasts, small lymphocytes, plasma cells, and eosinophils in the tissue spaces between the congested venous sinuses. The blotchiness was emphasized chiefly by the very dense nuclear structure of the many scattered groups of erythroblasts. The groups of small lymphocytes and plasma cells were more numerous here than in normal spleens and were scattered throughout the red pulp of the organ, suggesting active transformation of plasma cells from small lymphocytes, as shown by Bloom (1928) and Hu and Ch'in (1933). Occasional mitotic figures among eosinophil myelocytes, also indicative of myeloid metaplasia, were found throughout the red pulp, but chiefly along the trabeculae and in the subcapsular zones. Mitotic figures of lymphoblasts were also found in scattered clumps of lymphocytes in the red pulp. The lymphoblasts of the red pulp were the same in structure as those found in the germinal centers of the follicles. The eosinophils accumulated in areas where there were many macrophages active in phagocytosis. There were increased numbers of neutrophils, and free reticular cells and eosinophils were seen migrating through the lining of the sinusoids. In some spleens there was an increase in the number of megakaryocytes, so that in some parts of the red pulp six to eight could be seen in a single field with a magnification of 2700 diameters. No trypanosomes or leishmania bodies were found in the spleens. Histologically, this general reaction of the spleen of *Peromyscus* accords almost exactly with the findings reported by Crowell (1923) and DeCoursey (1935) for the spleens of human infants who died of Chagas' disease.

The specific reactions of the spleens of each of the five mice were so different as to warrant individual description. In the 50-gram mouse, which received one inoculation and was killed on the 23d day, the spleen showed a pronounced reaction (pl. 21, fig. 22). The organ was about twice the normal size. The follicles were three or four times as large as normal, and the cellular changes in the follicles were as described above for the more acute reaction. The red pulp showed the largest number of megakaryocytes of any infected spleen, along with many large lymphocytes and small accumulations of erythroblasts. Small numbers of plasma cells were scattered throughout the red pulp.

The 43-gram mouse, which weighed 48 gm. just before its death, was given one inoculation and was killed 276 days (approximately nine months) later. The spleen was only slightly larger than normal, but showed larger germinal centers. The red pulp showed a heavier infiltration of free cells, with small groups of eosinophils as well as single eosinophils scattered along the trabeculae and through the subcapsular zones. There were also a few groups of erythroblasts scattered in the pulp.

The 41-gram mouse received one inoculation and was killed 24 days later. The spleen showed no increase in size and less change than that observed in any other animal. The follicles did not differ from the normal, but the red pulp, especially around some follicles, showed greater numbers of lymphocytes. Scattered throughout the red pulp were more erythroblasts and plasma cells than normal.

The 33-gram mouse had been given five inoculations in all, namely, one inoculation daily for five successive days, using one bug each time, and was killed on the 21st day following the first inoculation. The spleen was about twice normal size and the follicles were from three to five times as large as normal. The obliteration of primary nodules was more complete here than in any other mouse (pl. 21, fig. 21; pl. 22, figs. 25, 27). The red pulp showed the most noticeable blotching, from the presence of many compact accumulations of polychromatophylic erythroblasts, small lymphocytes, and plasma cells.

The 20-gram mouse, which was about two-thirds grown and but recently weaned, had been given a daily inoculation for five successive days, each inoculation being the gut content of two bugs. This mouse was killed on the 27th day after the first inoculation. The spleen was about normal in size. The follicles were enlarged, but not so much so as in either the 50- or the 41-gram mouse. They showed distinct secondary nodules. The primary nodules formed a narrow zone surrounding the germinal centers and were one or two cell layers thick in only a few places between the secondary nodules and the reticular membrane of the follicle. The red pulp showed accumulation of groups of free pulp cells, thus giving it a blotchier appearance than normal.

BONE MARROW

The work of Petri (1934) on white mice has been followed for identification of bone-marrow cells. Counts were made on sections of sternal marrow of 1000 free cells, exclusive of nonnucleated red blood corpuscles, to determine the proportion of megakaryocytes in the different animals. There were 5 per 1000 in the 34-gram normal mouse, 10 per 1000 in the 50-gram infected mouse, 6 per 1000 in the 41-gram infected mouse, and 13 per 1000 in the 33-gram infected mouse. Comparative counts of 500 cells from femur impression and streaked smears in a 41- and a 33-gram infected mouse showed no significant changes from counts on a 36-gram normal mouse.

LYMPH NODES

In a single lumbar lymph node approximately 0.5 mm. in diameter, from the 47-gram normal mouse, there were five or six follicles scattered about the

periphery of the gland just under the cortex. The dense masses of small lymphocytes which composed the periphery of the follicle stood out from the surrounding, more loosely constructed medullary tissue. Several follicles showed well-developed germinal centers composed chiefly of medium-sized and large lymphocytes with fixed and free reticular cells scattered throughout. The interfollicular tissue, composed mostly of small lymphocytes, with scattered medium-sized lymphocytes, plasma cells, macrophages, and occasional eosinophils, completely fills the rest of the gland with the exception of some areas in the medulla, where the lymphatic vessels anastomose before leaving the gland. The interfollicular spaces are here almost filled with reticular and lymphoid cells, as in the mesenteric lymph glands of white mice (Downey and Weidenreich, 1912). Inside the connective tissue of the capsule, as well as in the sinuses just under the capsule, were isolated mast cells or groups of mast cells. A few were found in the medullary cords near the center of the gland. This agrees essentially with the findings of Downey and Weidenreich (1912) for the mesenteric lymph glands of white mice, but they emphasize that there is great structural variability in individual lymph glands, even from the same animal.

In the 41- and in the 50-gram mouse, the inguinal lymph nodes were enlarged, and in the latter mouse the bronchial lymph nodes were also enlarged. In the 20-gram mouse there were two enlarged lumbar lymph nodes each measuring about 3 mm. in length. These nodes showed thirteen to fifteen active germinal centers scattered throughout the cortex and medulla of the glands. The primary nodules were obliterated in most of the follicles by the larger germinal centers composed of numerous mitotic figures scattered among the medium-sized and large lymphocytes, and numerous macrophages which were actively phagocytic. Where the primary nodules persisted, it was difficult to tell where the follicle ended and the interfollicular tissue began. The interfollicular tissue, composed chiefly of small and medium-sized lymphocytes, a few eosinophils, and plasma cells, completely filled the spaces between the follicles, except for scattered blood vessels and reticular cells. Mast cells, either singly or in groups, were found in the connective tissue of the capsule and trabeculae. Some isolated mast cells were found in the central parts of the glands in the sinuses and in the tissue between the sinuses. The changes observed here were similar to those observed by Crowell (1923) in the lymph nodes of an eight-month-old female mulatto infected with *T. cruzi*. He found that there were marked congestion, lymphoid hyperplasia, and active phagocytosis in the cervical, axillary, and inguinoceural nodes.

DISCUSSION

The normal blood picture of white mice has been thoroughly studied by De Kock (1931) and Petri (1933, 1934). No attempts were made by De Kock to subdivide the various cell types beyond lymphocytes, monocytes, neutrophils, eosinophils, and basophils, since he maintains that only these forms are recognizable by the three methods of tissue culture, supravital preparation, and May-Grünwald-Giemsa staining. Petri recognizes among the neutrophils, pro-

leucocytes, ring forms, and complicated nuclear forms. The writer's unsegmented neutrophils correspond to Petri's (pl. 1, figs. 1, 2, 5) proleucocytes. The ring forms of Petri (pl. 1, figs. 6 to 13), however, include the segmented and multilobulated forms of the blood of *Peromyscus*. The neutrophils of *Peromyscus* are less variable in structure than those of white mice. There are no neutrophils in this white-footed mouse comparable to Petri's complicated nuclear forms. The type *a* and type *b* eosinophils of white mice correspond to type *a* and type *b* eosinophils of the blood of *Peromyscus*. The basophil leucocytes here, in contrast to those of white mice, possess definite ring-shaped nuclei like the other granulocytes and have been grouped into types *a* and *b* like the eosinophils. Petri's differentiation of lymphocytes is essentially the same as used here. He characterizes the mononuclears as being of the same size as the human monocyte, yet morphologically they are more like the lymphocyte type, especially with respect to their protoplasm, and so he calls them "large mononuclear cells." The mononuclears of the blood of *Peromyscus* show less variability of nuclear shape than those of the white mouse. Loewenthal (1933, 1934) described the leucocyte types of the field mouse, *Arvicola arvalis*. His main cell types are essentially the same as those found in *Peromyscus*.

Michels (1931) states that in the blood of mammals polychromatic red corpuscles are as abundant as are the reticulocytes. The counts on the blood of *Peromyscus* do not verify this statement.

Before a discussion is given of the pathological findings, it would be well to compare available information with respect to the relative strengths of different strains of *Trypanosoma cruzi*. The California strain, as shown by Kofoid and Donat (1933*a*) and Wood (1934*a, b*), runs a rather mild course in all animals investigated. Only in a few laboratory animals were symptoms of infection noticeable. The trypanosomes first appeared in the blood of albino rats from the sixteenth to the twenty-seventh day after inoculation. No laboratory animals were permanently incapacitated in any way or died from the infection, but white-footed mice (*Peromyscus*) were most susceptible to infection. Zuccarini (1930), using four different South American strains, found that white mice were the most sensitive experimental animals. The incubation periods for the four strains ranged from five to twenty days, yet all strains were fatal to adult mice between thirty and ninety days after inoculation. Niño (1929) found that white mice were most susceptible to the Argentine strain. The incubation period ranged from six to forty-one days, the average being nineteen days. Most of the mice died after two months. Galliard (1929) has shown that mice may become infected and die without the appearance of trypanosomes in their peripheral blood. In all animals studied here, trypanosomes were found in the peripheral blood.

Emmanuel Dias (1934) has shown that *Trypanosoma cruzi* may infect man for as long as twelve years. He has also shown (1932*a*) that the minimum time for organisms to appear in the peripheral blood of dogs is forty minutes by the subcutaneous route and two hours, forty-five minutes by the peritoneal route when numerous organisms are injected. Rapid passage of trypanosomes into the blood stream was also noted after intramuscular inoculation and in-

tracerebral injection. Galliard (1929, 1930) maintains that in white mice the subcutaneous route of inoculation is preferable to the intraperitoneal, in view of the precocity and intensity of the manifestations that it produces. When Dias inoculated white mice with the intestinal contents of infected *Triatoma*, the initial blood phase, mentioned above, did not occur, because the organisms did not appear in the blood until the eleventh day. In one animal inoculated intraperitoneally with the intestinal contents of an infected *Triatoma protracta* (California strain), the author noted the appearance of trypanosomes in blood smears on the seventh day after inoculation, but in other *Peromyscus* the incubation period was longer.

Changes in the blood picture of Chagas' disease have been reported by Ezequiel Dias (1912), Mazza (1926*a*, *b*), Geoghegan (1929), MacRobert (1929), and Niño (1929). Dias' work is the most comprehensive hematological investigation on man. Owing to the high incidence of other parasitic infections in the territory studied, Dias had only nineteen cases limited to *Trypanosoma cruzi* infection alone. He compared findings of different cases with normal counts made by himself on persons free from parasites. His results show no anaemia, decreased hemoglobin and specific gravity, slight leucocytosis in acute cases, pronounced macrolymphocytosis in acute cases and recently infected cases, and generally an eosinophilia and light basophilia in old cases. The blood counts by Mazza (1926*b*) and Geoghegan (1929) on single human cases agree with the findings of Dias. MacRobert (1929) reports a high percentage of lymphocytes in the blood for a case with nervous symptoms.

Niño (1929) reports numerous observations on the blood of white mice and other laboratory animals infected with *Trypanosoma cruzi*, Argentine strain. White mice were most susceptible to infection and most of the infected animals died in about two months. There was an intense anaemia of the regenerative type which manifested itself by changes of chromophilia, appearance of normoblasts, and especially by the appearance of large numbers of red corpuscles with Howell-Jolly bodies. There was marked anisocytosis, and in some cases an appreciable poikilocytosis. During the course of the infection there was an initial lymphocytosis lasting from one to fifteen or twenty-five days after inoculation, followed by a polymorphonuclear neutrophil leucocytosis, which persisted or returned to a level approximately the same as for the lymphocytes until the death of the animal. In *T. cruzi* infections in dogs, Mazza's (1926*a*) work showed a lymphocytosis. The author's finding of a lymphocytosis agrees with those of Ezequiel Dias (1912) and Niño (1929). It is interesting that with the California strain the postlymphocytic neutrophil leucocytosis was not evident even up to thirty-six days after infection, whereas in Niño's experiments on white mice a neutrophil leucocytosis was generally evident twenty-five to thirty-five days after infection.

It has been noted that splenic enlargement in white mice, accompanied by myeloid metaplasia, is common in some infections (Jarotzky, 1908; Jolly, 1923; Simonds, 1925; McCoy-Hill, 1930). Definite hyperplasia of different cell elements of the spleen, especially the reticulo-endothelial cells, is a general accompaniment of infectious states. Dominici (1900*b*, 1901, 1921) and Maxi-

mow (1927) have noted that eosinophil leucocytes are readily mobilized at sites of such reactions, as has also been found in this investigation.

Enlargement of the spleen has been observed in guinea pigs infected with *Trypanosoma congolense* and *T. gambiense* (Laveran, 1908), in rats infected with *T. lewisi* (Marmorston-Gottesman, Perla, and Vorzimer, 1930), in guinea pigs infected with *T. equiperdum* (Poindexter, 1933), and in hamsters and rats infected with *Leishmania donovani* and *T. brucei*, respectively (Hu and Cash, 1929; Hu, 1931, 1933, 1934; Hu and Ch'in, 1933).

In *Trypanosoma cruzi* infections in white mice, Niño (1929) observed in the spleen of different animals hyperplasia of the connective tissue, thickening of the capsule, intense congestion, inflammation, fibrosis, hyperplasia of the lymphoid tissue, and in some spleens abundant blood pigment. A definite hyperplasia of the lymphoid tissue and marked increase in the cellularity of the interfollicular tissue was observed in some spleens of white-footed mice infected with the California strain.

Various peculiarities in tissues have been noted in infections with *Trypanosoma cruzi*. Souza-Campos (1929) found in infected dogs changes in the nuclei of the reticulo-endothelial cells, which contained numerous parasites. Torres and de Azevedo (1929a, b) found numerous multinucleated giant cells in the myocardium of the armadillo, and in the heart, thyroid, and kidneys of dogs experimentally infected from the armadillo. These giant cells showed numerous leishmaniform bodies of *T. cruzi* in their cytoplasm, which was bordered by a distinct membrane forming a true double-walled cyst. Mazza (1930) found these giant cell cysts in the heart and muscle fibers of the bronchi of the armadillo. Villela and Dias (1934a, b) have reported necrotic ulcers of the bones and joints, external genital organs, mouth, skin (hair follicles, epidermis, and dermis), and digestive mucosa (stomach, intestine) in dogs experimentally infected from the armadillo. No such changes were observed in the hematoplastic tissues of mice infected with the California strain.

Locatelli (1929) observed extensive changes in the megakaryocytes of the bone marrow of guinea pigs infected with *Trypanosoma equiperdum* and *T. brucei*. In animals which died three or four months after inoculation, he found nearly all the nuclei of mature megakaryocytes in pycnosis and there were very few young forms in the marrow. In animals which died a short time after inoculation, numerous mitoses of megakaryocytes were found in all stages of division and there were many young forms in the marrow. The effect of the infection on the bone marrow, according to Locatelli, was a stimulation of the mature megakaryocytes toward degeneration and of the young forms toward maturation. Although some increase of cells was noted in the sternal marrow of *Peromyscus*, there was no evidence of degeneration of the nuclei or mitotic figures of the megakaryocytes.

The most important single cellular response in the spleen of *Peromyscus* to the action of the California strain of *Trypanosoma cruzi* is the active proliferation of cells of the reticulo-endothelial system, as described above. Hu (1931, 1934), Marmorston-Gottesman, Perla, and Vorzimer (1930), and Poindexter (1933) have pointed out that the reticulo-endothelial cells are the chief sites

of response in other trypanosomiasis. Cash and Hu (1929), Hu and Cash (1929), and Hu (1933) have shown that in hamsters infected with kala azar, the most characteristic change is a reticulo-endothelial hyperplasia, but here the action is evidently both mechanical and toxic, since numerous macrophages are found whose cytoplasm is filled with parasites. Some strains of *T. cruzi*, especially in dogs, seem also to give this type of reaction, as shown by Souza-Campos (1929) and Emmanuel Dias (1932b). As Dias points out, there exists in such instances an infection blockade of the reticulo-endothelial system.

SUMMARY

1. The normal blood of the white-footed mouse (*Peromyscus*) includes orthochromatic and polychromatic red corpuscles, neutrophil, eosinophil, and basophil leucocytes, lymphocytes, mononuclears, and platelets.

2. An increased number of large lymphocytes in the peripheral blood from fourteen to thirty-six days after inoculation was produced by experimental infection with *Trypanosoma cruzi*, California strain.

3. The eosinophil leucocytes were decreased in number from twenty-six to thirty-six days after inoculation.

4. The histology of the normal spleen of *Peromyscus* is described.

5. Infected mice showed enlargement of the spleen up to twice normal size.

6. Enlarged bronchial, lumbar, and inguinal lymph nodes were found in infected animals.

7. Hyperplasia of the lymphoblasts and lymphocytes of the follicles in the spleen, resulting in increase in size of the follicles from three to five times normal, was observed in animals killed twenty-one to twenty-seven days after inoculation.

8. Myeloid metaplasia was noted in the spleen of some mice, involving increase of erythroblasts and eosinophil myelocytes.

9. Megakaryocytes were increased in sections of spleen and sternal marrow of infected animals.

10. Hyperplasia of the reticulo-endothelial cells of the spleen was produced in white-footed mice by experimental infection with the California strain of *Trypanosoma cruzi*.

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EXPLANATION OF PLATES

All figures of plate 20 were drawn by Dr. Fae D. Wood with the aid of a camera lucida, \times 2100. All cells, except the trypanosomes in figure 2, and figure 15, are from normal blood. The cells in figures 1 and 3 were stained with Giemsa's blood stain after treatment with Knoll's fixative, and benzidine solution; that in figure 4 was stained with Giemsa's blood stain after treatment with Knoll's fixative, and benzidine and copper sulphate solutions. All other cells were stained with May-Giemsa.

The photomicrographs of plates 21 and 22, which were made from slides stained with Delafield's hematoxylin and eosin, were taken by Mr. J. E. Gullberg.

PLATE 20

Fig. 1. Multilobulated type neutrophil showing varying intensity of oxydase reaction in neutrophil granules (benzidine solution only).

Fig. 2. Red blood corpuscle, blood platelets, and *Trypanosoma cruzi*.

Fig. 3. Type *a* eosinophil showing pronounced oxydase reaction (benzidine solution only).

Fig. 4. Segmented type *a* neutrophil after oxydase reaction (benzidine and copper sulphate solution).

Fig. 5. Multilobulated type neutrophil showing numerous granules.

Fig. 6. Segmented type *a* neutrophil showing few granules.

Fig. 7. Segmented type *b* neutrophil showing many granules.

Fig. 8. Unsegmented type neutrophil showing few granules.

Fig. 9. Type *a* eosinophil.

Fig. 10. Type *b* eosinophil.

Fig. 11. Type *a* basophil.

Fig. 12. Type *b* basophil.

Fig. 13. Mononuclear showing fine chromatin meshwork.

Fig. 14. Mononuclear showing coarse and fine chromatin meshwork.

Fig. 15. "Macrolymphocyte" from blood of mouse infected with *Trypanosoma cruzi*.

Fig. 16. Large lymphocyte with irregularly shaped nucleus.

Fig. 17. Small lymphocyte.

Fig. 18. Medium-sized lymphocyte with granular inclusions.

Fig. 19. Medium-sized lymphocyte.



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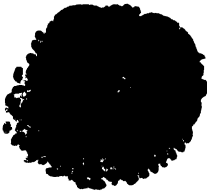
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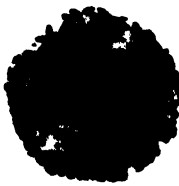
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PLATE 21

Fig. 20. $\times 30$. Spleen from 34-gram normal mouse, showing small size of follicles and distinct zonation of primary and secondary (germinal center) nodules. Note the extent of the interfollicular tissue and its appearance, owing to its low cellular content.

Fig. 21. $\times 30$. Spleen from 33-gram infected mouse, showing increase in the size of the follicles tending toward obliteration of the primary nodules. Note the distinctness of the marginal zone surrounding the follicles and the sharp contrast with which this zone is set off from the interfollicular tissue. The scattered groups of developing erythroblasts, eosinophil myelocytes, small lymphocytes, and plasma cells give the blotchy appearance to the interfollicular tissue.

Fig. 22. $\times 30$. Spleen from 50-gram infected mouse, showing enlargement of the follicles, with increased cellularity of the interfollicular tissue.

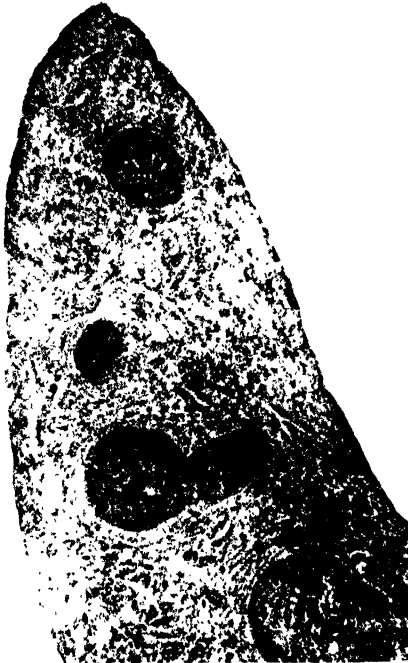
Fig. 23. $\times 275$. Interfollicular tissue of the spleen of figure 20, showing scattered groups of deeply stained small cells (mostly erythroblasts).



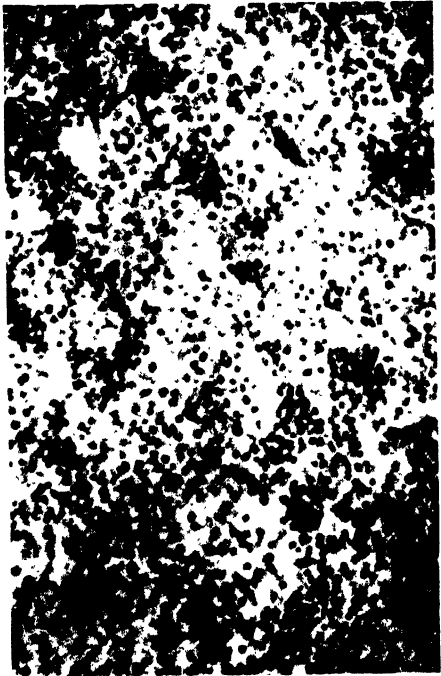
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PLATE 22

Fig. 24. $\times 125$. Follicle from spleen of 34-gram normal mouse, showing active germinal center (secondary nodule) completely surrounded by the primary nodule, which in turn is surrounded by a more or less distinct marginal zone which merges at various places with the interfollicular tissue.

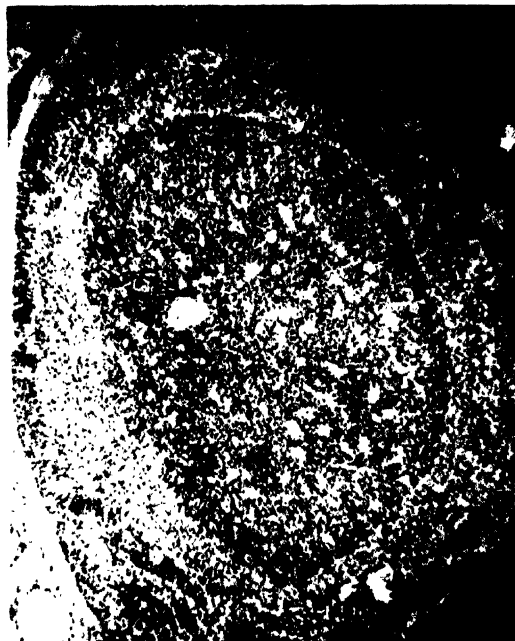
Fig. 25. $\times 125$. Follicle from spleen of 33-gram infected mouse, showing partial obliteration of the primary nodule owing to the great activity of the cells of the germinal center. Observe the distinct marginal zone sharply separated from both the primary nodule and the interfollicular tissue. Note the giant cells in the interfollicular tissue.

Fig. 26. $\times 530$. Part of follicle of figure 24, showing character of the nuclei of cells of different zones, from the germinal center (top) to interfollicular tissue (bottom). Note the nuclei of medium-sized and large lymphocytes and of a few reticular cells in the germinal center, the deeply stained nuclei of the closely packed small lymphocytes of the primary nodule, and the nuclei of the small and medium-sized lymphocytes in the marginal zone. The borders of some sinuses are visible in the interfollicular tissue.

Fig. 27. $\times 530$. Part of follicle of figure 25, showing character of the nuclei of cells of different zones, from the germinal center (top) to the interfollicular tissue (bottom). Note the few scattered cells of the primary nodule, the distinct broad marginal zone, and the increased cellular content of the interfollicular tissue.



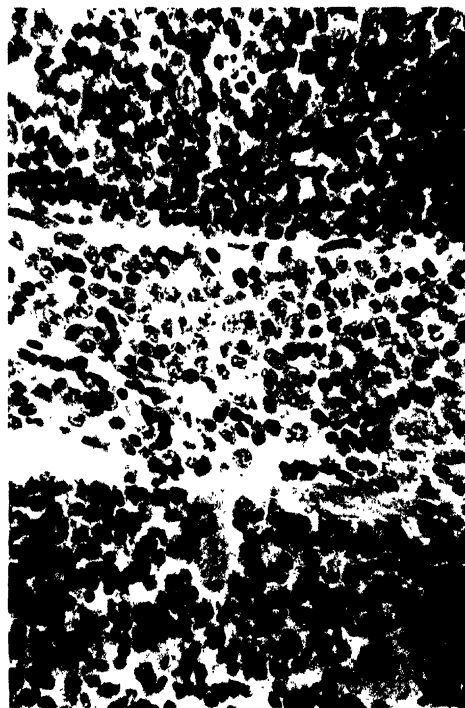
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**A NEW TREMATODE,
PARAMPHISTOMUM CASTORI SP. NOV.,
FROM CASTOR CANADENSIS BAILEYI
NELSON, FROM MARY'S RIVER, NEVADA**

BY

CHARLES A. KOFOID AND JAMES T. PARK

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A NEW TREMATODE, PARAMPHISTOMUM
CASTORI SP. NOV.,
FROM CASTOR CANADENSIS BAILEYI NELSON,
FROM MARY'S RIVER, NEVADA

BY

CHARLES A. KOFOID AND JAMES T. PARK

A NUMBER OF TREMATODES from the digestive tract of the American beaver were collected by Mr. A. E. Peterson while he was associated with an expedition of the California Museum of Vertebrate Zoölogy into northeastern Nevada in the summer of 1935. The expedition was under the direction of Professor E. R. Hall. Through the courtesy of the men just named we have been able to examine the trematodes, and find them to be undescribed. No animal food was found in the stomach contents of the beaver, which contained only willow bark. The subspecies *Castor canadensis baileyi* is limited to the drainage basin of the Humboldt River. The flukes were taken from the caecum and large intestine.

Paramphistomum castori sp. nov.

Figs. 1a, b.

Description.—Body elliptical or spindle-shaped in outline, 7.5–19 (11) mm. in length by 3–7 (6) mm. in width; cuticula smooth; oral sucker subterminal, globular in outline, 1.21–2.39 (1.93) mm. in diameter; posterior sucker near posterior end of body, 1.33–3.18 (2.39) mm. in diameter; oesophagus muscular, 0.23–0.71 (0.42) mm. in length; oesophageal gland present; intestinal caeca large, with slightly wavy outline, extending nearly to posterior end of body; genital pore near the level of intestinal bifurcation, median or submedian; genital atrium very short, covered with cuticula; sinus sac muscular; cirrus sac, cirrus, and prostate cells present; ejaculatory seminal vesicle within the sinus sac, with inner surface covered with cells similar to prostate cells; seminal vesicle anterior to anterior testis, constricted into two or three successive subequal spheroidal regions; testes tandem, median, bilobed in young specimens, in older animals with as many as 15 elongated, pointed, subequal lobes, largely intercaecal; ovary very small, globular or subglobular, median or submedian, posterior to testes, 0.18–0.71 (0.57) mm. in diameter; shell gland posterior to ovary; Laurer's canal present, opening dorsally; coils of the uterus never extensive, always intercaecal, dorsal to testes, with large amount of spermatozoa found in sectioned specimens in anterior part; metraterm present; eggs yellowish brown, not operculated, 0.12–0.16 by 0.07–0.10 mm.; vitellaria well developed, lateral, extending from anterior level of posterior testis to near posterior end of body.

The character of the excretory system was not determined, owing to the condition of the specimen.

Type specimen in collections of University of California; paratypes deposited in United States National Museum.

Relationships.—*Paramphistomum castori* is similar to *P. cervi* (Zeder, 1790) in possessing tandem testes. However, it may be distinguished from the latter by the following characters: (1) The testes are highly branched instead of lobed. (2) The vitellaria extend to the anterior level of the posterior testes instead of to the posterior region of the oral sucker. (3) The sinus sac is present instead of being absent.

Discussion.—The presence of a definite cirrus in three specimens sectioned brings up the question whether or not the genital structures in *P. castori* should be named according to the nomenclature used in *P. cervi* (Zeder) by Fiscoeder (1903) and in *P. explanatum* (Creplin, 1849) by Maplestone (1923). Although no concrete evidence has been found, the position of the metraterm and the muscular structures of the genital region seem to indicate

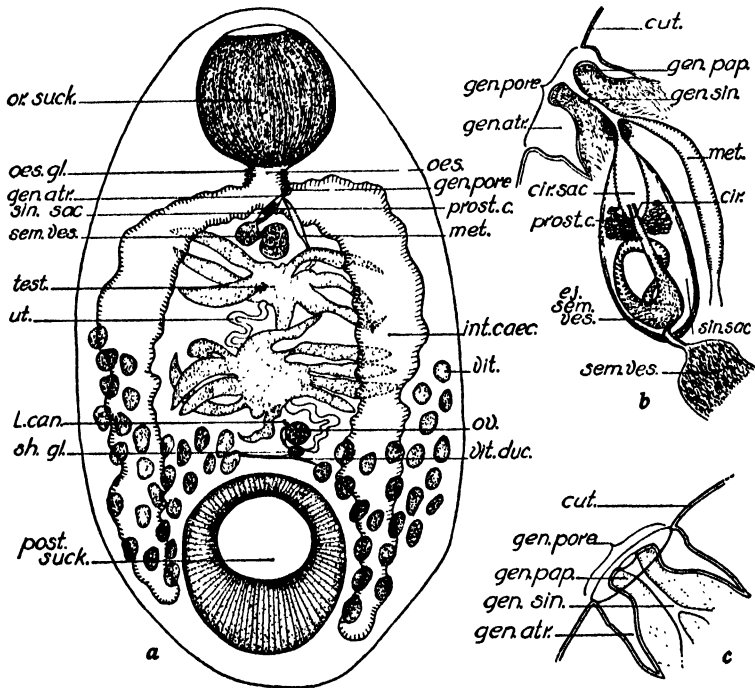


Fig. 1a. Ventral view of *Paramphistomum castori* sp. nov. $\times 10$.

Fig. 1b. Genital structures of the same. $\times 77$.

Fig. 1c. Genital opening. $\times 70$.

Abbreviations: *cir.*, cirrus; *cir. sac.*, cirrus sac; *cut.*, cuticula; *ej. sem. ves.*, ejaculatory seminal vesicle; *gen. atr.*, genital atrium; *gen. pap.*, genital papilla; *gen. pore*, genital pore; *gen. sin.*, genital sinus; *int. caec.*, intestinal caecum; *L. can.*, Laurer's canal; *met.*, metraterm; *oes.*, oesophagus; *oes. gl.*, oesophageal gland; *or. suck.*, oral sucker; *ov.*, ovary; *post. suck.*, posterior sucker; *prost. c.*, prostate cell; *sem. ves.*, seminal vesicle; *sh. gl.*, shell gland; *sin. sac.*, sinus sac; *test.*, testis; *ut.*, uterus; *vit.*, vitellaria; *vit. duc.*, vitelline duct.

that the cirrus is protrusible at the time of self- and cross-copulation, at which time the cirrus sac is obliterated. If this be so, the duct called the hermaphroditic duct in *P. cervi* and *P. explanatum* should be called the genital sinus in *P. castori*. When the protrusion of the cirrus occurs, the cirrus sac becomes a part of the sinus sac. However, the histological structure of the inner and outer layers still distinguishes these two sacs since the posterior part of the sinus sac is composed of several muscular layers and the cirrus sac proper lacks comparable muscles. The muscular sinus sac may be easily overlooked or interpreted merely as the muscle fibers around the male genital structures.

The pars prostatica, sphincter muscle, and pars muscosa in *P. cervi* and *P. explanatum* are homologous to the cirrus sac, cirrus, and ejaculatory seminal vesicle of *P. castori*.

Fischhoeder (1903) and Travassos (1934) do not mention the cirrus sac in *Paramphistomum*, and Stiles and Goldberger (1910) state that it is absent in this genus. Its presence in *P. castori* will require a modification in the generic characters in this respect.

SUMMARY

Paramphistomum castori sp. nov., from the American beaver, *Castor canadensis baileyi* Nelson, from Mary's River, Elko County, Nevada, is described. It differs from *P. cervi* (Zeder) in having branched instead of lobed testes, vitellaria extending only to anterior testis instead of to oral sucker, and presence of a sinus sac. The presence of a cirrus requires a modification of the characters of the genus.

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